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ALLERGY: A DERMATOLOGIST'S REMINISCENCES AND SPECULATIONS*†‡

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THE very day that I received the great honor of your invitation to deliver the Prosser White Oration I began searching for a suitable topic. For I realized that I must hope that the merits of my theme might hide my own inadequacies and the imperfections of my presentation.

Dr. Corsi, Dr. MacKenna, and Dr. Schneider-Green were kind enough to help me in my selection, and agreed that some remarks on a dermatologist's view of Allergy might prove a happy choice. The subject is a fascinating one, and certainly big enough for me to hide behind.

DEFINITION

What is "allergy"? It has always seemed to me that in order to be employed to its full value, the designation "allergy" must be used in the original sense proposed by von Pirquet when he coined the term in 1906.

It was von Pirquet's explicit purpose to include under allergy all specific acquired alterations in capacity to react which are produced in living organisms by exposure to living or inanimate agents. The quality of specificity here depends upon the circumstance that the exposure to the given agent produces a change which can be made manifest solely when the organism or tissue experiences subsequent exposure to the very same immunologic entity, that is, to the original agent or an "immunologic relative" thereof.

ALLERGY DOES NOT NECESSARILY MEAN HYPERSENSITIVITY

There is at present a rather widely-held and unfortunately stubborn notion that allergy must needs mean an increased susceptibility or an acquired hypersensitivity. Von Pirquet indicated his full realization that the new term would be unnecessary if it were to describe merely acquired hypersensitivity. Quite on the contrary, von Pirquet and most of the earlier workers, like Schick, recognized the far-reaching and fundamental relationships, though not necessarily

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complete identities, between the mechanisms and phenomena of specifically acquired increased sensitivity and of specifically acquired decreased sensitivity, of specific resistance or immunity. They were fully aware that these apparently opposite directions of change can be based on the same or closely similar biologic reactions (immunologic mechanisms); may be mixed up together, accompany or follow one another in the same animal or tissue; and may even result from one and the same exposure to the same agent. It was the recognition of these intimate relationships which impelled von Pirquet to propose "allergy" as an inclusive and unifying term to embrace all the forms of specific acquired alterations, regardless of the direction of the changes. By supplying such a new and nonprejudicial term he hoped to spare future workers the necessity of speaking of an individual as becoming "hypersensitive" to tubercle bacilli when he has actually become relatively immune to the disease (I refer you to the results of B.C.G. vaccinations as modern substantiations), and to prevent the nonsensical designation of a guinea-pig as "immunized" to a substance when the poor animal has really become so the opposite of immune that exposure to a single drop of a 1:1,000,000 dilution of the agent concerned can suffice to bring about his sudden demise!

HARMFUL VERSUS BENEFICIAL EFFECTS

It is, I believe, all too often forgotten that the pioneers in allergy recognized the reaction of allergic hypersensitivity to be, in and of itself, harmful—yet also, at least at times, beneficial; or better stated, that the very reaction of allergic hypersensitivity can contain both beneficial and harmful components.

Certainly none could deny—either now or formerly—that under von Pirquet's definition there must be included such unequivocally beneficial allergies as antitoxic immunity. But I submit that, just as recognized by the original workers, there is today ample evidence that even the reaction of allergic hypersensitivity is not necessarily entirely harmful. Let me therefore here express my inability to understand or follow the persistent discussions as to "whether allergy is harmful or beneficial." It is neither, and it is both, it is preponderantly one or the other, depending upon one's point of view and upon the site, the speed, the quality, and other circumstances of the particular reaction. A gumma, a local Arthus' phenomenon, a strong and necrotic reaction to tubercle bacilli or tuberculin, a kerion or granuloma Majocchi due to fungi, and many other such lesions may be mentioned as reactions indubitably based upon high degrees of local allergic hypersensitivity. Are these lesions harmful or beneficial? No one can answer this question without first requiring replies to many others:

Where is the reaction occurring? If the gumma or the other reaction is of the skin, it is probably ultimately beneficial to the host. There is, at very least, the mechanical walling-off of the infection, the local destruction of microorganisms, and the eventual casting-out of the necrotic masses, together with the remains of the offending agents. But if this same gumma or other allergic reaction takes place in the wall of the aorta or in the respiratory center of the medulla, death of the host may well result from the allergic inflammation,

necrosis, and slough. If a severe allergic reaction is in and of the eye, there may be blindness; if of the bone, a fracture; but if of the glabrous skin, only an unimportant scar—perhaps with some depigmentation or hyperpigmentation. Therefore, when the question arises whether this or that type of allergic response or reaction of hypersensitivity is harmful or beneficial, no correct answer can be given until the questioner has supplied the particulars of exactly where the reaction is taking place.

Moreover, before attempting to reply to the original general question one must also require the answer to the query: Beneficial or harmful to what? To the host as a whole? Or to the tissues at the site of reaction? Or to some local function of the organ? Surely I need not labor this point by repeating the above examples, all showing that a reaction of allergic hypersensitivity always does some harm locally, even though it may, in the long run, serve most useful purposes for the individual as a whole.

ALLERGIC DISEASES (DUE TO LIVING OR INANIMATE AGENTS) CONTRASTED WITH TOXIC DISEASES

Allergic hypersensitivity is not only unexceptionally, to some degree, harmful to the tissues at the site of reaction, it is indeed the quite obvious sine qua non of allergic diseases in the usual narrower sense, and, in my opinion, the sole known mechanism of many an infectious disease not generally recognized as allergic.

That the allergic reaction is the principal basis for disease in hay fever, in many cases of hives or in eczematous contact dermatitis, can be taken for granted. But I find that the statement that this holds equally true in such diseases as tuberculosis, fungous infections, leprosy, and syphilis—and perhaps even in smallpox and vaccinia—usually elicits a demand for supporting evidence.

The first such item of evidence which may be adduced is the fact that many common infectious agents, such as tubercle bacilli, lepra bacilli, treponemes of syphilis, fungi pathogenic to the skin, or the virus of vaccinia have never been shown to possess or elaborate a toxin, nor any poisonous substance having the power directly and primarily to damage or destroy human cells or tissues (or, indeed, tissues of any animal). All obtainable extracts of these microorganisms are so harmless to the previously unexposed individual that no reaction or damage ensues when large quantities are injected or otherwise administered to the not previously exposed infant, man, or woman, or to the noninfected guinea pig or other animal. (A state of "nonreaction" which M. Leider and I have recently designated "native or original anergy."*) But once let an adequate exposure to the microorganism have taken place and a suitable incubation period have elapsed, then will administration of even the minutest amounts of the originally harmless extracts almost inevitably produce prompt damage. Moreover, it is generally at about the very time when the skin sensitivity to the specific extracts first becomes demonstrable that the first characteristic signs of the infectious disease make their appearance, and not before. These facts, together with all

*A Study of B. C. G. Vaccinations. Presented at the Society for Investigative Dermatology, Atlantic City, June, 1949, J. Invest. Dermat. 13: 249, 1949.

other evidence available today, support the thesis that the characteristic tissue reactions and the most important features of such common and devastating diseases as tuberculosis, syphilis, leprosy, fungous infections, and many others are induced by, and may even be the sole consequences of, the specifically acquired sensitivity of the host tissues to the allergenic constituents of the living agents concerned.

This chain of events stands in sharp contrast to the happenings in such diseases as diphtheria, tetanus, and the toxic manifestations of scarlet fever, chancroid, or furunculosis, in which all the evidence today indicates that the microorganisms elaborate or possess substances which are primarily damaging, substances which are types of poisons, intrinsically and of themselves harmful to the host tissues, without the necessity for any preceding exposure or induced specific alteration in the capacity to react. In these principally "toxic" diseases there is, of course, no interval required for sensitization to develop, and therefore no necessity for a true "incubation period" of sensitization. Quite the reverse of what holds for a principally allergic disease, such as tuberculosis, in the toxic diseases, like diphtheria, the specifically acquired alteration in the capacity to react is not usually a sensitization, but bears the opposite sign, that is, it is in the direction of lessened sensitivity, in the direction of acquired resistance or immunity. And this specific acquired immunity to toxins, this development of antibodies known as antitoxins, this undeniably beneficial alteration is just as much an allergy in von Pirquet's sense as is any acquired specific sensitivity or hypersensitivity.

TABLE I. DIRECTION OF ALTERATION

GROUP I PRINCIPALLY ALLERGIC DISEASES		REACTION OF INDIVIDUAL NOT SUSCEPTIBLE OR NOT EXPOSED	EXPOSURE(S) PRODUCE:	REACTION OF INDIVIDUAL SUSCEPTIBLE AND EXPOSED
INFECTIOUS	NONINFECTIOUS			
B.C.G., T.b., fungi, lepra, syphilis, vaccinia, etc.	Occupational and contact dermatitis, certain drugs, plants, insect bites, etc.	0	Specific alteration in capacity to reaction (allergy)	+
GROUP II PRINCIPALLY TOXIC DISEASES				
INFECTIOUS	NONINFECTIOUS			
Diphtheria (Schick tox.) Scarlet fever (Dick tox.) Tetanus (tox.)	Certain snake, bee, spider, other insect poisons, etc.	+	Specific alteration in capacity to reaction (allergy)	0

I have now sketched not only the two principal directions of allergic changes, but what I believe to be the two most common (but not the only) mechanisms whereby pathogenic agents (living or dead) can produce disease. Perhaps Tables I-III will serve to illustrate this statement.

There are, of course, very many combinations and transitions between these two types of dissimilar pathogenic effects shown in the tables. Thus, in certain diseases, such as scarlet fever, staphylococcic infections, e.g., furuncles, chancroid,

and even in diphtheria,* as modern studies progress, one can more and more clearly discern and separate the effects of the toxic component and of the allergenic sensitizing components of the microorganisms.

TABLE II. INCUBATION PERIOD, REACTION TIME, AND HEALING TIME

FIRST EXPOSURE TO:	TIME TO FIRST CHARACTER LESION	HEALING TIME
T.b., fungi, sera, simple chemicals, (neoarsphenamine, paraphenylenediamine, dinitrochlor, benz., phenylhydrazine) drugs (penicillin, sulfonamides, etc.).	Approximately 5 to 21 days' "incubation period" (not repeated)	Slower (often several weeks)
Re-exposure to same agents	Usually a few hours to a few days' "reaction time" (remains fairly constant)	Quicker (approx. 10 to 14 days)

TABLE III. PRESUMABLY PRINCIPALLY TOXIC AND PRINCIPALLY ALLERGIC DISEASES (CONTRAST BETWEEN TIMES TO FIRST APPEARANCE)

TOXIC DISEASES	USUALLY LESS THAN 5 DAYS; OFTEN ONLY SEVERAL HOURS TO 4 DAYS AFTER INFECTION
Diphtheria	2-8 days
Scarlet fever	1-5 days
Tetanus (except spores?)	4-21 days (plus)
ALLERGIC DISEASES	**USUALLY 5-6-10-21 DAYS + AFTER INFECTION**
T.b.—B.C.G.	5-6-10-21 days
Fungi	5-6-10-21 days
Syphilis	10-42 days
Smallpox	9-15 days
Vaccinia	5-6 + days
Serum disease	5-6-10-21 days
Simple chemicals	5-6-10-21 days

BENEFICIAL EFFECTS OF LOCAL REACTIONS OF HYPERSENSITIVITY EVEN IN ALLERGIC DISEASES SENSU STRICTIORI

If we now had several hours instead of only one, I would have liked to discuss with you how, even in allergic diseases in the narrowest sense, even in hay fever, asthma, and hives, and above all, in the allergic contact dermatitis and occupational eczemas, the local tissue reaction has a great deal of the beneficial about it. That weeping eyes, running noses, sneezing, and coughing serve to wash off, blow off, and expel noxious invaders is clear enough, but perhaps physicians and investigators do not think sufficiently of how the allergic skin lesion repels attacks, and how in so doing the skin sacrifices part of itself; of how the vesicle of eczema lifts off and holds off, and finally casts off the potentially damaging agents which have fixed themselves to the skin's surface; of how the bulla or blister interposes a lake (or rather a river) of diluting fluid between the attacked hard, horny, "expendable" surface tissue and the subjacent softer and more vulnerable living structures. Nor do we always sufficiently regard how the later weeping and oozing of eczema wash off what may remain of damaging

*Thus in diphtheria it appears that the "false" or "pseudopositive" Schick reactions are due to the skin's acquired sensitivity to the sensitizing, thermostable allergens of the bacillus, and, in contrast to the true positive or toxin reactions, can be elicited even with heat-inactivated toxin or toxoid.

agents after the blister top has been cast off; nor note how the acanthosis and keratosis of chronic eczema provide a greater, thicker, tougher protective cover; and of how even the itching and scratching (admittedly almost always finally harmful) may have original beneficial components, in that they lead to brushing off or scraping away the attacking agents.

THE COMMON ALLERGENS ARE PROTEAN RATHER THAN PROTEIN

Let us now go on to another facet of our subject, namely, the wide variety and diversity in the nature of the substances now known to be capable of causing allergic changes. The simple chemicals causing drug eruptions or occupational and other forms of contact dermatitis or eczema, the contact allergens in the oleoresinous fractions of plants, the so-called "protein" allergens of hay fever, up to and including the large molecules of the polysaccharides and proteins and unknown sensitizing substances of bacteria, fungi, spirilli, and virus, as well as the complex toxins and venoms—all these have already been mentioned as capable of causing the specifically acquired alterations in capacity to react.

I don't know how it is now here in England, but in the United States it has been a slow process to root out the idea that true allergic reactions and allergic diseases can be produced only by proteins (e.g., by foods or pollens or other inhalants).

I recall quite vividly that I had but shortly arrived in the United States from my studies under Br. Bloch, and Josef and Werner Jadassohn, Max Jessner, Wm. Frei, R. L. Mayer and others, and was beginning to practice dermatology together with my great friend and teacher, Fred Wise. I was not only callow in the practice of dermatology and ignorant of the customs of physicians in the United States, but was saturated with a sort of vicarious wisdom and experience absorbed spongelike from my great teachers. I was called in by a general practitioner to see a young man in his teens, presenting a fever of 104° F., and a diffuse morbilliform rash (but not measles—the family physician who had called me had seen the young man through a typical attack of measles several years previously).

I took a careful history, focused upon drugs, and found that several doses of a preparation containing aspirin had been taken about seven days before the onset of the present illness. When I stated my opinion that the disease we were seeing was in all probability a drug reaction from salicylates, the general practitioner looked upon me, more in sorrow than in anger, and spoke to the following effect: "But, young man, how can you be so brash and so ignorant of even the rudiments of medicine and diagnosis? Don't you realize that this must be an infection? That the fever proves beyond doubt that we are dealing with an infection?"

I was never again called in by this particular physician. But a few years later we met by chance, and he told me that the patient we had examined together had recovered rapidly, and that he had, a few weeks later, again taken aspirin, with the prompt recurrence of the previous picture of both rash and fever. The physician had then been convinced that I had guessed right, but had felt disinclined to call me again after the fatherly dressing down he had

given me at our first meeting. But even then, after the demonstration ad oculos, this really excellent physician, practicing in New York City in the 1930's, thought this case to be a curiosity, a bizarre and unusual happening, a negligible exception. He was by no means convinced that morbilliform eruptions, combined with high fever, should be regarded as common and characteristic allergic reactions to simple nonliving chemicals without benefit of a living—or at least a protein—constituent. It was not until years later, when sulfonamide reactions opened the eyes of the medical profession at large, that the recognition of the great variety of drug reactions, so long well known and accepted by dermatologists (Morrow, 1887; Fox, 1890; Jadassohn, 1896), acquired universal acceptance, and became added to the store of general medical knowledge.

ADVANTAGES OF USING SIMPLE CHEMICAL ALLERGENS IN IMMUNOLOGIC EXPERIMENTS

If, as we now perceive, simple chemical allergens can and do reproduce most of the phenomena of many infectious diseases; and if, as we now recognize, many important infectious diseases are based principally upon allergic sensitization to the products of the infecting agents, is it not reasonable to conclude that many laws governing immunities and susceptibilities to the more complex and variable living agents may perhaps best be elucidated through experiments making use of simple allergens? For by this device the investigator will generally avoid the sources of error inherent in the uncontrollable, unpredictable and ofttimes, undiscoverable variations in the chemicophysical composition of the living agents. Is the suspension of treponemes or tubercle bacilli which is being used by the investigator today really the same as that of yesterday? And will that of tomorrow be like today's? What I am trying to convey is that, in experiments designed to elucidate the principles of immunology, it is often more exact, more direct, and simpler to use an allergenic solution of a mercurial salt, of formalin, arsphenamine, or a dye, than to use treponemes, virus, bacteria, or "soups" extracted from plants, foods, or inhalants. And it is in this particular effort at clarification through simplification that dermatologists have often been the leaders, as documented by the studies of such men as J. Jadassohn, Cranston Low, Br. Bloch, R. Prosser White, W. Jadassohn, W. Frei, R. L. Mayer, Arthur Whitfield, A. Tzanck, and many others of our colleagues who have contributed so mightily to immunologic knowledge through their studies of skin sensitization to simple chemicals.

VARIATIONS IN SUSCEPTIBILITY TO EXPERIMENTAL SENSITIZATIONS AND THEIR IMPLICATIONS FOR HUMAN HEALTH AND DISEASE

I well recall some of my own small parts in such endeavors to find eternal truths by means of external applications and simple chemicals. At J. Jadassohn's clinic in Breslau, in 1927, Frei had succeeded in sensitizing the skins of a certain percentage of guinea pigs and human volunteers with a single intracutaneous injection of a dilute solution of neoarsphenamine; R. L. Mayer had succeeded in sensitizing guinea pigs' skin with applications of paraphenylenediamine. At the same time, W. Jadassohn at Zürich had achieved and was

studying the guinea pig's skin sensitization to phenylhydrazine. Together with Frei and Mayer, I had been conducting experiments in the endeavor to ascertain some of the factors causing the wide variations in susceptibilities and resistances of guinea pigs to cutaneous sensitization by neoarsphenamine and paraphenylenediamine.

When I arrived in New York City and visited Karl Landsteiner at the Rockefeller Institute, I found that this most eminent immunologist of our time had tried to repeat our European work with simple chemicals (arsphenamine, paraphenylenediamine, and phenylhydrazine), and had failed to sensitize a single guinea pig! Landsteiner and all his able co-workers were most polite, but (mark this well!) were all highly skeptical as to the possibility of experimentally sensitizing the skin of any laboratory animal or man with a simple chemical.

I then proceeded to roll up my sleeves and to "show them." I used the same techniques, dosage, etc., I had employed at Breslau and Zürich, the same age and weight, size and color of guinea pigs. Despite repeated attempts and numerous series of experiments, not one of the Rockefeller Institute animals could be sensitized. I need not tell you that "my face was red." Then began a great carrying to and fro of guinea pigs, young and old, pregnant and unborn, of neoarsphenamine, of glassware, of syringes, of needles, of water, and of fodder between the Universities of Breslau and of Zürich and the Rockefeller Institute in New York City. Finally, after about four years, Drs. Landsteiner, Wm. Frei, Philip Levine, Merrill Chase, R. L. Mayer, and I all agreed that it was an indisputable fact that even American guinea pigs could be skin-sensitized with a simple chemical like neoarsphenamine. Moreover, we were all equally convinced that the susceptibility to skin sensitization on the part of guinea pigs varied in a tremendous range. In some of the series in New York there were zero sensitizations, while the incidence rose to 50 per cent or more in other series—still far below the high rates of 80 per cent to 90 per cent achievable during the winter in Breslau! We began to try to elucidate the reasons for these variations. As Merrill Chase then showed, selective breeding from easily sensitized guinea pigs can produce families of animals highly susceptible to such sensitization and vice versa. Thus, hereditary factors in the guinea pigs are important—but surely not the whole story.

Something in certain kinds of green fodder, the vitamin C content of the diet, the season in which the experiment takes place, even the manufacturer's batch of the drug, are also of influence. As R. L. Mayer, B. Oser, and F. Simon, and I have shown, each of these factors can be influential in raising and lowering the susceptibility to sensitization, but neither alone nor in combination do they account for all the variations observed. In other words, we have gathered much knowledge, but are yet very far from knowing all or even the most decisive factors bringing about susceptibility or resistance to allergic sensitization in this instance.

I know you will all see the analogies between these laboratory observations with paraphenylenediamine and arsphenamine and that which is observed clinically in most human "allergic diseases" and in many human infections. One could, I believe, substitute the words "human being" for "guinea pig," and the

words "allergenic drug" or "occupational contact allergen" or "pollen" or "tubercle bacillus" or other allergenic microorganisms for "neoarsphenamine," and find that practically all the factors discovered as influencing resistance and susceptibility in the guinea pig-arsphenamine experiment have been implicated to some degree and at some time in the variable human susceptibilities to clinical diseases. Moreover, just as in the outlined dermatologic laboratory experiments, in human diseases also the great decisive factors which make for high susceptibility or adequate immunity of a species, a family or an individual, an organ or part of an organ—or even a group of cells, or a single cell within an organ—are still largely unknown. The fact that these problems can perhaps best be studied further through deliberate skin sensitizations with simple chemicals constitutes, to my mind, one of the greatest opportunities for modern dermatologic research in allergy.

THE ALMOST INEXHAUSTIBLE VARIETY OF ALLERGIC DISEASES. DRUG REACTIONS SUPPLANT SYPHILIS AS THE GREAT IMITATOR

If individual and species susceptibilities to allergic alterations are of almost infinite variety, and the causal agents of allergy are protean and diverse, both are surely no more so than the morphe and types of reaction, than the pictures of disease which may be evoked when specifically sensitized tissues meet their adequate allergenic stimulus. In the human skin there are innumerable examples of allergic, presumably allergic, and partially allergic reactions and diseases. Deserving of first mention are the three classic, often indubitably allergic, reactions: the urticarial, the tuberculin type, and the eczematous response, each of which can be produced at will by bringing the appropriate allergen to the sensitive skin by means of the suitable technique. In addition to these three, there are the fixed drug eruptions of several types: the "exanthematic" eruptions, with and without fever, with and without other systemic and visceral responses; the multiforme erythemas and nodose erythemas, the lichenoid, the vesicular, bullous, or pemphigoid reactions; and perhaps even such dermatoses as the acneiform and the fungating halogen eruptions, the purpuric responses to drugs and infections, and a multitude of others. In some of these instances the evidences of allergy, i.e., of preceding specific sensitizing mechanism, are clear; in others, the role of these mechanisms is unclear or even unlikely. In the unclear instances the possibility of other mechanisms of pathogenesis, such as summation or potentiation of the damaging effects of several subthreshold exposures or activation of dormant agents, must be borne in mind. Unfortunately, time does not allow me to consider the many different forms of these—sometimes at least partly allergic—summations, potentiations, and activations. But I would like at least to mention some of their many names, such as "synergism," "trigger mechanism," Koebner's "isomorphic response," "Sanarelli-Shwartzman phenomenon," "nonspecific flare-up," "metallergy," "parallergy," and "biotropism of Milian."

Of course the skin has no monopoly on variety in allergic diseases. Modern teaching emphasizes that allergic diseases can affect most organs and assume many guises. Once again these truths are clearly discerned through the study

of reactions to simple chemicals. Drugs can cause nondermatologic diseases, ranging from fever to psychotic and manic states, from hepatic disease to neuritis, from blood dyscrasias to cancers. And in many of these drug reactions allergy would seem to play some part.

It is therefore correct to say that today the great imitator syphilis has been to a large degree supplanted by the greater imitator "drug reaction."

The far-reaching practical implications of this fact were once expressed to me clearly when I visited Dr. L. Dienes, the pathologist of the Massachusetts General Hospital, and he said: "If, as you state, the manifestations of drug reactions can so closely mimic those of other diseases, then a good physician, when baffled by a disease of obscure genesis, should not so often say, 'Take this medicine,' but more often say, 'Now stop taking that medicine.' "

STUDIES OF THE SENSITIZING POTENTIALS OF CHEMICAL ALLERGENS AND THEIR PROCLIVITIES TOWARD AFFECTING CERTAIN SITES IN THEIR OWN PARTICULAR MANNERS MAY SHED LIGHT ON MANY BASIC PHENOMENA OF INFECTIONS

It is important for my thesis here to note that dermatologic studies have shown that certain drugs are much more likely than others to produce allergic disease, and possess what Rostenberg, Jr., and the speaker have called a high "sensitizing potential." Phenolphthalein, arsphenamine, and salicylates are common causes of allergic reactions; other drugs, for example, cascara sagrada, probably self-administered by just as great a number of people, are nevertheless essentially negligible as elicitors of allergies. Moreover, particular drugs and chemicals are inclined to produce their own particular forms of allergic disease, and other allergenic chemicals, other forms—e.g., salicylates tend to produce urticaria and angioneurotic edema; sulfonamides, scarlatiniform, morbilliform, or nodose eruptions; mercury and quinine, eczematous reactions; arsphenamines, exfoliating dermatitis; antipyrine and phenolphthalein, their characteristic fixed polychromatic plaques; and penicillin, either generalized urticaria or dysidrosis-like or "phytid-like" manifestation of the hands and dermatophytosis-like eruptions of feet and groins.

The reasons why some substances are highly allergenic, and others scarcely so or perhaps not at all, still almost completely escape us. Above all, we remain almost entirely ignorant of the why and wherefore of the tendency of a particular substance to produce its particular type of allergic disease. These are fertile fields, and most challenging ones for study. The great unifying concept of allergy provides the hope that by discovering some of the reasons for the relative sensitizing potentials and the selective proclivities of particular inanimate chemical agents to produce particular forms of disease, one may disclose certain of the most fundamental reasons for the predisposition, resistance, and characteristic responses to many infections. Perhaps through studies with simple allergens, investigators may one day learn why the treponeme of syphilis is allergenic to man and causes its human disease, while the *Treponema microdentium* and *Treponema macrodentium* remain harmless; or why the gonococcus produces its usual lesions in the urethra, cervix, or tubes, and sometimes in the eye, but never

in the mouth or throat, while the meningococcus spares the urethra and prefers to attack the meninges; or why diphtheria is generally a disease of the nasopharynx and throat rather than of other equally exposed sites. Thus the very nature of disease, its location and form, and its epidemiology, the susceptibilities and immunities of species, of strains, of families, of individuals, of systems, of particular organs—yes, even of particular tissues or cells within organs—may be in some way, and perhaps in some rather decisive way, related to the problems of the varying susceptibilities to the allergenic effects of simple chemicals.

DO THE FINDINGS IN ALLERGIC ECZEMATOUS RESPONSES PROVE THE EXISTENCE OF EPIDERMAL AND, THEREFORE, OF EPITHELIAL SHOCK TISSUES? SOME IMPLICATIONS OF THIS CONCEPT, INCLUDING SPECULATIONS ON CARCINOGENESIS

Another way of saying that a particular organ or part of an organ is for unknown reasons especially susceptible to sensitization and allergic disease is to speak of "shock tissues"—a term which is much and glibly used, and which to my mind again states a problem without giving solutions. You all know that in the urticarial and the tuberculin-type responses it is recognized that the regions of the upper cutis, in particular the vascular plexus, are the shock tissues, and that in referring to the eczematous form of allergic response it is fashionable to say that the epidermis is the shock tissue.

Many great experimenters like Br. Bloch, Cranston Low, Civatte, Haxthausen, Mom, and others have concerned themselves with this problem without, in my opinion, bringing it to a decisive issue. Some days I am inclined to believe that the epidermis is really the shock tissue; some days I tend to believe that the epidermal reaction, though always observed early in the eczematous process, is nevertheless quite likely secondary to something which has happened below in the immediately subjacent layers of the cutis (nerves? blood vessels?). It depends largely upon what I have just been reading or seeing, or to whom I have just listened.

The decision is important. Here is a place for studies with tracer substances, for tissue cultures of sensitized epidermal cells, for experiments in symbiosis, for skin transplants, for visualization with phase- and electron microscopes. I know of no problem more deserving of our best wits and the application of our most highly developed experimental techniques. For if the epidermis, the epithelial element itself, is indeed a shock tissue, then the possibility is at hand for scrutinizing a great many baffling old problems from new immunologic and allergic points of vantage. If the highly differentiated epidermal cell can be specifically sensitized, then there is no reason why the cell of the islet of the pancreas should not become a shock tissue, its reactions leading to some forms of diabetes; nor any reason why particular nerve or brain cells, circulating blood cells or marrow cells should not become shock organs, and their allergic reactions lead to psychic and nervous diseases, leukemias, anemias, or agranulocytosis.

Perhaps more important and thought-provoking still is the speculation that the epithelium of many an organ could perhaps be specifically sensitized in such a manner that the repeated subliminal (subclinical) allergic reactions would con-

stitute a mechanism of carcinogenesis, and would eventually lead to the mysterious epithelial transformations which result in cancer. Though this is admittedly no more than an hypothesis, I have long felt that it was a good one for dermatologists and immunologists to begin teamwork upon. And in this opinion so competent an authority as the late James Ewing agreed when he went so far as to tell me that it was the most original and constructive idea on carcinogenesis which had come to his attention in many years.

Surely it falls particularly to dermatologists to occupy themselves with these problems. For it is not by chance that so many carcinogenic agents and mechanisms have been discovered through studies of cancer and precanceroses of the skin. It is easy to document this statement with a most impressive list which might start as follows: The scrotal soot and pitch cancers of Sir Percival Pott's "climbing boys"; the arsenical warts and arsenical cancers of miners and others; Bowen's and Paget's precanceroses, the aniline cancers, mule spinner's, and oil cancers; the light cancers; the scar and kangri burn cancers of Kashmir; the experimental tar cancers of Yamagiva and Ichikawa (1913), of Br. Bloch (1927, 1932), Bloch and Dreifuss (1921, 1922) and Bloch and Widmer's (1926) separation of the coal tar fractions most carcinogenic to the skin; Kennaway, Cook, and co-workers' isolation of the chemical cutaneous carcinogen, benzpyrene; the virus-induced skin tumors of Peyton Rous and malignant papillomas of Richard Shope; and Miescher's and others' experimental roentgen ray cancers. Even this fragmentary list will serve to show how consistently dermatologic manifestations and observations have led to the most fundamental discoveries in the field of cancer. Both past achievements and contemporary investigations indicate that the time is here for planned cooperative experiments on the possible relations between eczematous (epidermal ?) sensitization and experimental carcinogenesis in the skin. Despite the wide differences, there are numerous, seemingly more than fortuitous, resemblances between the two processes.

There is, for example, the phenomenon that many known carcinogens are simple chemicals, sometimes identical with, and sometimes bearing astonishing similarities to structures known to be eczematogenous allergens. Then there are the atypical and "precancerous" epithelial proliferations, produced both by carcinogens and occasionally by eczematogenous allergens when the latter meet the sensitized tissue. More important still, there is the common feature of the incubation period; for just as a certain minimum of time must elapse before the eczematogenous allergen can produce its characteristic epithelial change, just so an irreducible minimum of time is required before the first carcinomas appear—no matter how frequent or how intensive are the applications of carcinogen.

Another analogy between the two processes is discernible in the astonishingly variable species, family, organ, and local susceptibilities to the specific action of chemical carcinogens; they act much like the often gene-determined but also dietarily, seasonally, and otherwise influenced variations in the susceptibilities to the specific effects of eczematogenous allergens.

Still another finding which suggests the possible carcinogenic potentialities of eczematogenous allergens is R. L. Mayer's (1948) recent demonstration of the strong affinity for nuclear structures evidenced by certain notoriously eczematogenic dyes, and their influences upon the enzymatic respiratory processes. But to my mind, the experiments pointing most unmistakably to the possible immunologic basis of some forms of carcinogenesis are those performed in 1943 by Simpson and Cramer (1944) in Cowdry's laboratories at the St. Louis Free Skin and Cancer Hospital and Washington University Medical School (Cowdry, 1947). These investigators showed that 3 per cent methylcholanthrene in wool fat (anhydrous lanolin) is not itself carcinogenic, but that its application so sensitizes the skins of susceptible strains of mice that they develop unusually large numbers of cancers with quite exceptional rapidity when at some much later time they are exposed to the carcinogenic action of methylcholanthrene in benzene. While these, and all other items I could submit at present, would not suffice to prove basic relationships, they do encourage and even oblige to further ventures in experimental carcinogenesis, utilizing in concert the knowledge and skills of the disciplines of dermatology and immunology.

ORGAN-SPECIFIC BODY-OWN ANTIGENS; SPECULATIONS AS TO THEIR POSSIBLE ROLES IN CARCINOGENESIS, IN MANY CHRONIC DISEASES AND IN DERMATOSES, INCLUDING THE DERMATERGOSES

If sensitization of epithelia can be conceived as a possible mechanism in some forms of carcinogenesis, what is the nature and provenance of the postulated sensitizing allergenic carcinogens?

In oil and tar and pitch cancers, in aniline and miners' cancers—in all such, the carcinogens come from without. But is it not conceivable that in the case of other cancers, cancers of the breast, prostate, uterus, liver, or other viscus, endogenous substances—for example, steroid hormones or other sterol derivatives—can sometimes act as sensitizing and carcinogenic allergens?

This question leads me to the last of my themes, namely, of how certain phenomena of allergy have, in recent years, somewhat breached the barrier of Paul Ehrlich's concept of horror autotoxicus. For it has now been proved beyond doubt that certain autogenous substances (perhaps only when slightly altered by infection or physicochemical trauma) can on occasion act as antigens, produce antibodies, and through these elicit disease in the very organs from which they are derived. I have elsewhere (Sulzberger, 1940) discussed at some length the important studies in this field up to the year 1940, and shall here merely mention a few of these.

They include the experimental production of cataract by antilens antibodies (Burky and others); kidney disease by antikidney antibodies (Masugi, Swift, Smadel, Schwentker, Cavelti, and others); brain and spinal cord disease by antimyelin antibodies (Rivers, Schwentker, Kopeloff, and others); and leukopenias by antileucocytic sera; as well as demonstrations of specific sensitization even to body-own hormones such as insulin (Tuft, Criep, Loveless, and others), pituitrin

(F. Simon and others) and estrogens and androgens (B. Zondek and others). I would, however, like to spend a few more minutes on some of the pertinent studies which have been published since 1940.

In 1948, K. Lange and co-workers were able to demonstrate circulating anti-kidney antibodies in a relatively high percentage of patients with glomerulonephritis; in 1947 Kerr and Cavelti reported experiments showing that damage can be produced not only in the kidney, but perhaps also in the heart and blood vessels by their respective organ-specific antibodies.

As a very last reference to Dermatologic Allergy I hope you will not consider it presumptuous of me to quote one of my own statements (Sulzberger, 1940, p. 424):

"And to return to dermatologic allergy, is it not possible that damage to the skin—alteration and liberation of skin constituents (either through infection or through physical agents or through reactions to allergens)—may create and liberate skin-antigen, which in turn produces skin-specific antibodies, which in turn produce reactions in the skin itself, wherever and whenever skin antigen is made available and accessible to their action? Would not this hypothesis explain—perhaps better than all others—the recognized fact that after an eczematous response has been produced in one area, there often follow months and years during which, not only areas of exposure, but also almost all other skin sites react with pathologic responses to all manner of forces and substances previously innocuous and previously tolerated?" (Compare Gear, 1946.)

I hope you recognize why I have asked your indulgence for this "autogenous" quotation. It is solely because there is such close kinship between this speculation on my part and the earlier astute clinical observations on autosensitization eczema by a late distinguished member of this Society, Arthur Whitfield. I therefore believe that while they are surely worthy of note by all practitioners and investigators, the latest, and I hope very promising, experiments on autogenous skin sensitization may be of special interest to the present audience.

In one of the newer experiments to which I refer, Hecht, Weil, and I (1943) were able by a few technical tricks to induce rabbits to produce precipitins to their own skins, and showed that in the rabbits with these circulating antiskin antibodies mechanical trauma and chemical irritation of the skin produced local reactions far in excess of, and perhaps also different from, the reactions elicited in control animals by the equivalent trauma and irritants. Dr. Weil tells me that he has recently repeated these experiments with confirmatory results.

A second series of experiments which has some bearing here is that carried out by Harold F. Blum, Rudolf L. Baer, and me (1946), with results which suggest that in urticaria solare due to wave lengths of less than 3700 A° the substance(s) to which the patients are hypersensitive and to which they have circulating Prausnitz-Kuestner antibodies is some autogenous derivative, produced not only by irradiation of the skin of the patient, but in every normal person by the action of the rays in question. The difference between these patients and the normal persons is apparently the fact that the patients become allergic to the agent regularly produced by the rays, while normals do not.

* * * * *

I hope that I have now to some degree succeeded in showing that the term "allergy" was not meant to apply to only a few diseases, like hay fever and eczema, or to be restricted to exceptional persons and their peculiar idiosyncrasies. On the contrary, for the concept to prove useful, one must think of allergy as a widely encountered, fundamental mechanism of biologic *adaptation,* through which the individual adjusts to, and finally triumphs over, the onslaughts of other species and of his environment. Thinking of allergy in this way enables one to understand why skin specialists have contributed—and, I venture to predict, always will contribute—richly to the study of allergic phenomena. For the skin is ever the first in protection, the first in adjustments to the environment, and, therefore, also the first in allergic adaptations. The skin meets all comers, disposes of almost numberless specialized protective devices, often heroically sacrificing parts of itself in its defense of the individual as a whole. Please bear with me for just a moment while I contrast the brave skin with the pusillanimous pancreas or the languid liver. When compared with the exposed cutaneous organ, these and their companion viscera lead sheltered lives, far behind the lines of battle. They are shrouded in everlasting darkness, protected from every change of temperature or humidity, and, like the fetus in utero, bathed, fed, and suspended in an almost shockproof and well nigh always sterile chamber. They rely upon the skin to shield them from most of the onslaughts of the outer world. This the skin does, reacting and adjusting its reaction, allergically or otherwise, as the strategy demands; setting up its successful defenses against the light and heat, cold and dampness, against the attacking hosts of infectious agents, the hordes of insects, the pressures and frictions, the sharp objects and the blunt, the chemical irritants and poisons, and the already countless yet ever-growing numbers of medicinal, household, cosmetic, clothing, occupational, and other allergens of civilization. To my way of thinking, it is not just a fortuitous happening that Robert Prosser White could write so great a book on the *dermatergoses,* and that there are no texts on *hepatergoses* or *pancreatergoses.*

It is true that this exposed position of the skin is not an unmixed blessing to the dermatologist, whose diagnoses and therapy are often rendered difficult by the ever-changing influences of the outer world upon the skin. For just like those coming from within, each of these external influences inevitably modifies the skin's diseases and responses. Yet we are indeed fortunate among physicians in being able most clearly to observe and precisely to study both that which happens spontaneously and that which we deliberately produce in our special organ. And it is this which places us under the most stringent *noblesse oblige* to contribute unflaggingly to the advances of medicine as well as affording us "unequalled opportunities for the study of morbid processes."*

* * * * *

Here at my closing I perceive that, just as I feared, I have fallen far behind my distinguished predecessors in bringing you classical allusions and enlivening

*Quoted from Jonathan Hutchinson. No dermatologic oration, nor, indeed, any serious discussion of dermatology's past and future would be entirely complete without reference and due deference to the great Sir Jonathan.

anecdotes. However, the inspiration of the occasion and the stimulus of my favorite topic have combined to lead me to distinguish myself by what may be a too liberal indulgence in speculations.

Mention of the ways in which men can distinguish themselves always brings to my mind one of my vivid recollections of my father—namely, that he was given to sneezing a most amazing number of times in quick succession. He never stopped with two or three sneezes, but usually went on to twenty or thirty explosive sternutations in a row. If some sound or movement of those about him attracted his attention, this forced him to break off in mid-career, and apparently filled him with feelings of frustration and physical distress. Therefore, my mother and all of us thirteen children had learned to preserve rigid silence and immobility while father was sneezing.

Often when my father had come to the end of his attack I have heard my mother ask, "Ferdinand, what do you think it is that makes you sneeze like that?" This scene being laid in the preallergy era, my father could not say, "I must be allergic to something," but had to find some other excuse. Although he had distinguished himself in many ways, and received much recognition, he would always explain his distinctive sneezing by saying, "A man must distinguish himself in *some way!*"*

Just a few days ago I sat upon the other shore of the Atlantic Ocean, on the beach at Atlantic City, trying to gather my wits and fearing that I would soon "distinguish" myself by my complete lack of the voice and the vocabulary, the education and the inclination for oratory. As I thus sat looking out to sea toward England, a close formation of four jet-propelled planes flashed like silver missiles across the blue sky before my eyes, going so incredibly fast that they were disappearing in the misty horizon to my left even while I yet thought to hear their whistling, throaty roar coming toward me from my right.

Seeing them made me realize that my presence here might not be distinguished solely by the emission of these poor sounds and speculations, but perhaps also by at least one bright new circumstance.

To the best of my knowledge, I am the first American speaker to come to this Oration and these shores, not carried by ship, but borne on swift strong wings, high over the conquered barrier of the vasty ocean.

Perhaps this is a good omen; I hope so. Perhaps the speculations and flights of fancy today, with their weak and poorly stuck-on wings, may yet, like those of fabled Icarus, spur future men to better, stronger flights; and thereby help our coming colleagues to soar across the barriers of ignorance and error, and speed them as they rise to battle the vile and ancient enemies of man.

REFERENCES

1. Bloch, Br.: Schweiz. med. Wchnschr. **8**: 1218, 1927.
2. Bloch, Br.: Cancer Rev. **7**: 65, 1932.
3. Bloch, Br., and Dreifuss, W.: Schweiz. med. Wchnschr. **2**: 1033, 1921.
4. Bloch, Br.: Arch. Derm. Syph., Wien. **140**: 6, 1922.
5. Bloch, Br., and Widmer, F. E: Arch. Derm. Syph., Wien. **152**: 529, 1926.
6. Blum, H. F., Baer, R. L., and Sulzberger, M. B.: J. Invest. Dermat. **7**: 99, 1946.
7. Cowdry, E. V.: J. A. M. A. **135**: 408, 1947.

*"Ein Mensch muss sich durch Etwas auszeichnen."

8. Fox, T. C.: Brit. J. Derm. **2:** 327, 1890.
9. Gear, J.: Tr. Roy. Soc. Trop. Med. & Hyg. **39:** 301, 1946.
10. Hecht, R., Sulzberger, M. B., and Weil, H.: J. Exper. Med. **78:** 59, 1943.
11. Jadassohn, J.: Verhandl. d. deutsch. dermat. Gesellsch. **5:** 103, 1896.
12. Kerr, W. J., and Cavelti, P. A.: Tr. A. Am. Physicians **60:** 264, 1947.
13. Lange, K., Gold, M. M. A., Weiner, D., and Simon, V.: J. Clin. Investigation **28:** 50, 1949.
14. Mayer, R. L.: Proc. Soc. Exper. Biol. & Med., New York **68:** 664, 1948.
15. Morrow, Prince A.: Drug Eruptions, New York, 1887, William Wood & Company.
16. Pirquet, C. von: München. med. Wchnschr. **53:** 1457, 1906. English translation in Sulzberger's Dermatologic Allergy, p. 433.
17. Simpson, W. L., and Cramer, W.: Cancer Res. **5:** 5, 1944.
18. Sulzberger, Marion B.: Dermatologic Allergy, Springfield, Ill., 1940, Charles C Thomas, Publisher.
19. Yamagiva, K., and Ichikawa, K. (1913): Cited by Yamagiva, Katsusaburo: Virchows Arch. f. path. Anat. **233:** 235, 1921.

LOCAL ORGAN HYPERSENSITIVITY TO AUTOGENOUS ANTIGENS

I. Experimental Production of a Pneumonitis*†

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THE subject of autoantigenicity has, in the last fifteen years, assumed an increasing importance. Autoantibodies have been found in various human diseases. Animal experimentation has given weight to the concept of sensitization to one's own tissues.[1-31] The experimentally produced lesions were usually obtained in animals generally sensitized with organ or tissue extracts to which bacteria or other foreign antigens were added as adjuvants. The mechanisms involved were modified Arthus phenomena[9] and, presumably, the reaction of induced autoantibodies with the homologous organ.[18-31]

The purpose of the experiments reported here has been to study the autoantigenicity of: (a) a partly hydrolyzed autogenous substance (hydrolyzed autoserum); (b) normal body components (skin and liver extracts) placed in an abnormal environment within the same organism; and (c) an excretory product (urine) also placed in an abnormal environment.

A technique of local organ hypersensitivity was applied to the rabbit's lung; the production of an allergic pneumonitis was the criterion used to determine the antigenicity of the various autogenous products tested. No foreign antigens were added.

MATERIALS AND METHOD

Materials:

All experiments were performed on young adult rabbits weighing approximately 2,000 grams. Most of the rabbits used were albinos.

Three different autogenous allergens were employed.

(A) Hydrolyzed Autoserum.—The blood was obtained from the ear vein of the rabbit. After separation of the serum, hydrolysis was achieved in the following manner: 4 c.c. of serum were diluted with 8 c.c. of distilled water and 0.6 c.c. of 10 per cent sulfuric acid were added. This mixture was heated for one hour at 80° C. in a water bath. As a result of this treatment, a mixture of the normal proteins and the products of their partial hydrolysis (proteoses, large polypeptides) was obtained. The solution was neutralized to pH 7 with sodium hydroxide and then filtered through a Berkefeld filter. Sterile vials were filled with the solution to be used immediately, or stored in the ice box at 5° C. for further use on the same rabbit.

(B) Autogenous Urine.—The rabbit's urine was obtained aseptically from the bladder by puncture after laparotomy under general ether anesthesia and was injected slowly to the same rabbit, usually while the animal was still under anesthesia.

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†Preliminary reports of this work have appeared. [32-34]

(C) Saline Extract of One Organ Used to Sensitize Another Organ.—A piece of tissue was resected, rapidly washed in saline to eliminate the excess of blood, cut into small pieces, ground in a mortar with physiologic saline and sand, then blended for 2 to 4 minutes in a Waring blendor and centrifuged. The supernatant material was used immediately after filtration through Berkefield or Seitz filters. Two extracts were prepared in this way; a skin extract prepared from the same rabbit on which the experiment was made and a liver extract prepared from another rabbit.

A parallel series of experiments was run in which exogenous antigens (horse, bovine, and human serum) were used, with the purpose of comparing the antigenic power of endogenous and exogenous material.

Method:

The lung was sensitized by a single transthoracic injection of a small amount of antigen, using a 22-gauge needle, length one inch, introduced in the fifth or sixth intercostal space, at about the level of the posterior axillary line, avoiding any unnecessary trauma. The needle was inserted until contact with the lung tissue was felt, then advanced one more millimeter and the antigen was injected slowly.

After an interval of 2 to 9 weeks, the shock injection was given intravenously. The amount of antigen used for the shock injection was always larger than the amount used for the sensitizing injection. The amount of antigen injected and the time interval in the various experiments are shown in the tables.

After the shock injection, the animals were studied for signs of shock. In order to follow the evolution of the lesion the animals were killed, by injection of air unto the ear vein, at intervals of 1, 24, 48, 72, and 96 hours after the shock injection. Immediately after death a gross examination of the lungs, heart, liver, spleen, kidneys, peritoneum, and pleura was performed. Histological studies of the lungs were made for all the sensitized animals and most of their controls. The usual hematoxylin and eosin method of staining was used. In a number of instances, elastic and connective tissue stains were employed. Histological studies of other organs, especially the liver, were performed in certain cases.

Controls:

(A) In one series of controls, only the sensitizing injection was made, the animals being killed at different time intervals thereafter.

(B) In another series, only the intravenous injection of the antigen was made, the animals being killed at various time intervals after this injection. In a few cases, an injection of saline was made into the lung, followed after a suitable time interval by an intravenous injection of hydrolyzed autoserum.

(C) Another group of controls was used to demonstrate that the localization of the lesion in the rabbit's lung was not due to a special property of this organ. Experiments were performed in which the sensitizing injection was given in the eye, subconjunctivally or into the vitreous humor, or intracerebrally. The shock injection was given intravenously, as it had been for the animals sensitized in the lung. The animals were killed at various time intervals thereafter.

The details of the experimental procedure are shown in the tables. Animals sensitized in the lung with autogenous antigens are included in Table I; the various controls made with autogenous antigens are included in Table II; the animals sensitized in the lung with exogenous antigens and their controls are included in Table III.

TABLE I. EXPERIMENTAL (AUTOGENOUS ANTIGENS)
SITE OF SENSITIZING INJECTIONS: RIGHT LUNG

AUTO-ANTIGEN USED	NO. OF ANIMALS	EXPERIMENTAL PROCEDURE	TIME AFTER LAST INJECTION WHEN ANIMAL WAS KILLED	RESULTS*
Hydrolyzed autoserum	19	Sensitizing injection: 0.5-1 c.c. Time interval: 3-9 weeks Second injection: 2 c.c. intravenously	2 rabbits: 10-20 min.	1 rabbit: lesions, R lung ### 1 rabbit: lesions, R lung #
			14 rabbits: 1-3 days	5 rabbits: lesions, R lung ### lesions, L lung # in 2 rabbits 5 rabbits: lesions, R lung ## lesions, L lung # in 1 rabbit 2 rabbits: Lesions, R lung # 2 rabbits: no lesions
			1 rabbit: 10 days	Ascitic fluid in pleural cavity. No lesions in the lung
			1 rabbit: 20 days	No lesions
			1 rabbit: 40 days	No lesions
Autourine	3	Sensitizing injection: ½ c.c. Time interval: 18 days Second injection: 1-2 c.c. intravenously	20 min.	1 rabbit: lesions, R lung ## 1 rabbit: lesions, R lung # Some lesions, L lung 1 rabbit: lesions, R lung ### Some lesions, L lung
Rabbit liver	10	Sensitizing injection: ½-1 c.c. Time interval: 3-9 weeks Second injection: 1½-3 c.c. intravenously	1-3 days	4 rabbits: no lesions 2 rabbits: lesions, R lung # (1 rabbit: lesions, L lung) 2 rabbits: lesions, R lung ## 2 rabbits: lesions, R lung ### (1 rabbit: lesions, L lung)
Autoskin	2	Sensitizing injection: 0.5 c.c. Time interval: 15 days Second injection: 1½ c.c. intravenously	2 days	1 rabbit: lesions, R lung ## 1 rabbit: lesions, R lung #

*The results are based on the gross examination of the various organs. The organs examined are listed in the section "Material and Methods." Where no reference is made to the organ, there were either no lesions or lesions due to parasites, or caused by the air injection.

The lesions are classified as follows:

(a) No lesions (including those animals with only parasitic or caused by the air injection).
(b) #: one or a few small patches, or histologic lesion only.
(c) ##: one large patch (i.e., part of a lobe) or many large patches throughout the lung.
(d) ###: whole lung or one lobe.

TABLE II. CONTROL EXPERIMENTS (AUTOGENOUS ANTIGENS)

AUTO-ANTI-GEN USED	SITE OF SENSITIZING INJECTION	NO. OF ANI-MALS	EXPERIMENTAL PROCEDURE	TIME AFTER LAST INJECTION WHEN ANIMAL WAS KILLED	RESULTS
Hydro-lyzed auto-serum	Lung	8	Sensitizing injection: 0.5-2 c.c.	1 rabbit: 20 min.	Slight edema, local-ized at site of injection
				1 rabbit: 3 days	Small area of hepa-tization, sharply localized at site of injection
			No second shock injection	2 rabbits: 10 days	Very small and no reaction at site of injection
				2 rabbits: 20 days	No lesions
				1 rabbit: 40 days	No lesions
				1 rabbit: 60 days	No lesions
		2	Injection of 0.5 c.c. saline in R. lung Time interval: 5 weeks Second injection: 2 c.c. Hydrolyzed auto-serum, intrave-nously	2 days	No lesions
Hydro-lyzed auto-serum	Right eye (subcon-junctivally)	2	Sensitizing injection: ½-1 c.c. Time interval: 2 weeks Second injection: ½ c.c. intravenously	2 days	Congestion of con-junctival vessels of right eye; begin-ning 2 hours after shock injection, lasting 24 hours Normal lungs
Hydro-lyzed auto-serum	Right eye (vitreous humor)	2	Sensitizing injection: 0.1 c.c. Time interval: 4 weeks Second injection:	2 days	
			1 rabbit 2 c.c. intravenously		Retinal edema and rise in ocular ten-sion (right eye)
			1 rabbit 5 c.c. subcutaneously		No lesions, no change in ocular tension
					Normal lungs
Hydro-lyzed auto-serum	Brain	2	Sensitizing injection: 0.2 c.c. Time interval: 18 days Second injection: 2 c.c. intravenously	36 hours	Bilateral edema of brain Normal lungs
Hydro-lyzed auto-serum	Brain	2	Sensitizing injection: 0.2 c.c. No second shock injection	20 days	No lesions
Auto-urine	Lung (Right)	2	Sensitizing injection: ½ c.c. No second shock injection	7 days 18 days	No lesions No lesions
Auto-urine	No sensitiz-ing injec-tion	2	Intravenous injection only: 2 c.c.	20 min.	No lesions

TABLE II—CONT'D

AUTO-ANTIGEN USED	SITE OF SENSITIZING INJECTION	NO. OF ANIMALS	EXPERIMENTAL PROCEDURE	TIME AFTER LAST INJECTION WHEN ANIMAL WAS KILLED	RESULTS
Autourine	Eye (left) (subconjunctivally)	2	Sensitizing injection: ¼ c.c. Time interval: 20 days Second injection: 2 c.c. intravenously	2 days	Congestion of conjunctival vessels of right eye, beginning 1 hour after shock injection, lasting 24-36 hours Normal lungs
Rabbit liver	Lung (Right)	5	Sensitizing injection: ½-1 c.c. No second shock injection	18 days 5 weeks	No lesions
Rabbit liver	No sensitizing injection	6	Intravenous injection: 1½ to 3 c.c.	1 to 3 days	No lesions

RESULTS

1. *Control animals which had received only the intrapulmonary sensitizing injection.*—The sensitizing injection into the lung was followed by a focal congestive and edematous lesion which disappeared within from 10 to 15 days. Lungs studied from 2 to 6 weeks after this injection showed no gross or microscopic lesions.

2. *Control animals which had received only the intravenous injection.*—No reaction was elicited by the intravenous injection alone.

3. *Previously sensitized animals which, after a suitable time interval, had received an intravenous injection of the same antigen* showed a lesion in the sensitized organ, namely the right lung.

No lesions were found in other organs, except in a few cases (9/44) where similar lesions occurred in the other lung; these were as a rule much less severe than in the sensitized lung.

There was no evidence of shock following the intravenous injection. The pulmonary lesions appeared during the first hour. They were still present in animals killed 96 hours after the shock injection. Three animals killed on the eleventh, twentieth, and fortieth day showed no pulmonary lesions.

There were variations in the severity of the lesions in different animals even when the same antigen was used. A certain number of sensitized animals did not react at all to the shock injection. Of 44 animals, 11 showed no lesions,* 8 showed lesions classified as one plus, 11 showed lesions classified as 2 plus, and 14 showed lesions classified as 3 plus.

The severity of the lesions was not appreciably influenced when the time interval between the two injections was varied between 2 and 10 weeks, nor when the doses used at the two injections were varied within the limits used in these experiments.

*This number included the 3 rabbits killed at the eleventh, twentieth, and fortieth day.

TABLE III. EXOGENOUS ANTIGENS

ANTIGEN	SITE OF SENSITIZING INJECTION	NO. OF ANIMALS	EXPERIMENTAL PROCEDURE	TIME AFTER LAST INJECTION WHEN ANIMAL KILLED	RESULTS
Human serum	Lung (Right)	2	Sensitizing injection: ¾ c.c. Time interval: 2 weeks Second injection: 2 abd. c.c. intravenously	30 min.	1 rabbit: lesions, R lung ## 1 rabbit: lesions, R lung ### Also L lung #
Ox serum	Lung (Right)	2	Sensitizing injection: ¾ c.c. Time interval: 2 weeks Second injection: 3 c.c. intravenously	1 day	1 rabbit: no lesions 1 rabbit: lesions of both lungs, more marked in R lung ### than in left ##
Horse serum	Lung (Right)	6	Sensitizing injection: ½ c.c. Time interval: 3 weeks Second injection: 2 c.c. intravenously	20 min. to 4 days	3 rabbits: lesions, R lung ### 1 rabbit: lesions, R lung ## (very slight reaction L lung) 1 rabbit: lesions, R lung # 1 rabbit: no lesions
Horse serum	Lung (Right)	3	Sensitizing injection: ½-2 c.c. No second shock injection	4 days 12 days 14 days	No lesions No lesions No lesions
Ox serum	Brain	1	Sensitizing injection: 0.6 c.c. Time interval: 4 weeks Second injection: 2 c.c. intravenously	10 hours	Bilateral edema of brain Normal lungs
Horse serum	Brain	1	Sensitizing injection: 0.15 c.c. Time interval: 4 weeks Second injection: 2 c.c. intravenously	36 hours	Bilateral edema of brain Normal lungs
Horse serum	Brain	1	Sensitizing injection: 0.2 c.c. No second shock injection	4 weeks	No lesion
Ox serum	Eye (Right) (subconjunctivally)	3	Sensitizing injection: ¼-1 c.c. Time interval: 20 days Second injection: 2 c.c. intravenously	3 days	Violent congestion of conjunctival vessels of R eye In 1 rabbit slight congestion, L eye The lesions began to appear after 3 hours, lasted 2 days Normal lungs
Horse serum	Eye (Right) (subconjunctivally)	3	Sensitizing injection: ¼-1 c.c. Time interval: 20 days Second injection: 2 c.c. intravenously	2 days	Congestion of conjunctival vessels of R eye Slight congestion of conjunctival vessels of L eye The lesions began to appear after 2 hours, lasted 24 hours Normal lungs

As a rule the lesions observed in dark-haired rabbits were somewhat more severe than those observed in albino rabbits.

The lesions obtained with exogenous antigens were identical to those obtained with endogenous antigens.

4. *Control animals in which the sensitizing injection was given subconjunctivally, in the vitreous humor, or in the brain* developed lesions in the respective organs (conjunctivitis, retinal edema, brain edema) after the shock injection. No pulmonary lesions were found in these animals.

PATHOLOGY

Gross Pathology:

The distribution of the lesions in the affected lung varied. In severe cases, the entire lung or one or several lobes were involved in the reaction. Less severe reactions involved part of a lobe or were disseminated in foci throughout one lobe or the whole lung.

In animals killed during the first hour (Fig. 1), the affected areas showed congestion and edema with an increase in lung volume. In animals killed after the first 24 hours (Fig. 2), the affected areas appeared as dark, dull red patches, with sharp borders, often surrounded by small emphysematous zones; their consistency was firm and some fluid could be expressed on pressure. The involved parts were heavier than the rest of the lung when floated in water.

The control animals, including those which were sensitized in another organ (eye, brain) (Fig. 3), did not have lesions in the lungs, except occasional pinpoint red foci probably due to the air injection used to kill them.

Microscopic Pathology (Figs 4-8):

The lesions were located mainly in the blood vessels and alveoli. The differences in the pathological picture in different animals seem to be related to: (a) various degrees of severity of the vascular injury, and (b) differences in the age of the lesion; in general, the alveolar reaction preceded the visible arteriolar changes and the congestive and edematous features preceded the cellular infiltration.

Arteries.—Both the larger (muscular) and the smaller arterioles were involved.

Endothelium and lumen.—The endothelial reaction was characterized by swelling, desquamation, proliferation, and sometimes necrosis of the endothelial cells. It was seldom observed in animals killed during the first hour after the shock injection, was at a maximum in those killed at 24 and 48 hours, and decreased somewhat in the 72 and 96 hours' group. The lumen had often decreased in size, partly because of the endothelial proliferation and partly because of the increased thickness of the rest of the arterial wall. When blood was present, the number of leukocytes was increased and they were marginating.

Intima and media.—In the larger arterioles and especially in association with the more severe reactions of the endothelium, the intima was thickened. At

Fig. 1.

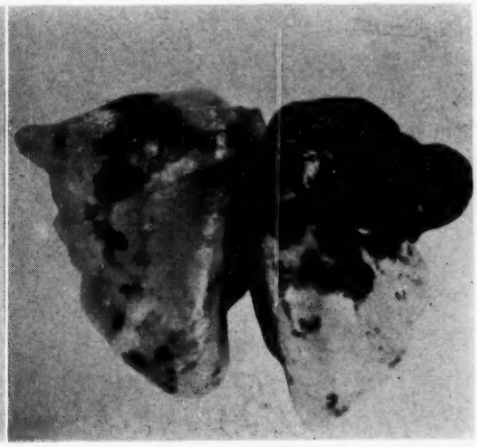


Fig. 2.

Fig. 1.—Lung sensitization. Lung reaction classified ###. Allergen: hydrolyzed autoserum. Sensitizing injection: 0.5 c.c. Time interval: 6 weeks. Shock injection: 1 c.c. Rabbit killed within one hour after shock injection.

Fig. 2.—Lung sensitization. Right lung reaction classified ###; left lung #. Allergen: hydrolyzed autoserum. Sensitizing injection: 0.5 c.c. Time interval: 6 weeks. Shock injection: 2 c.c. Rabbit killed 48 hours after shock injection.

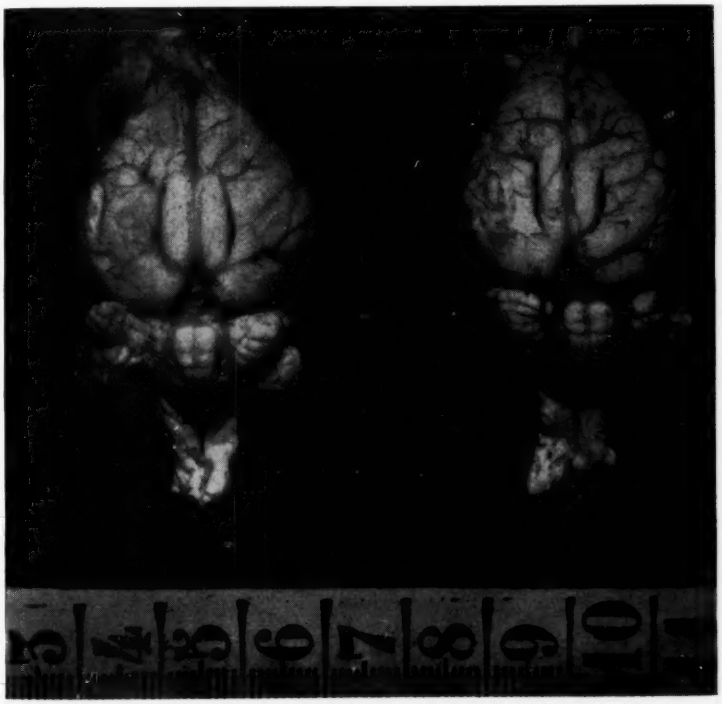
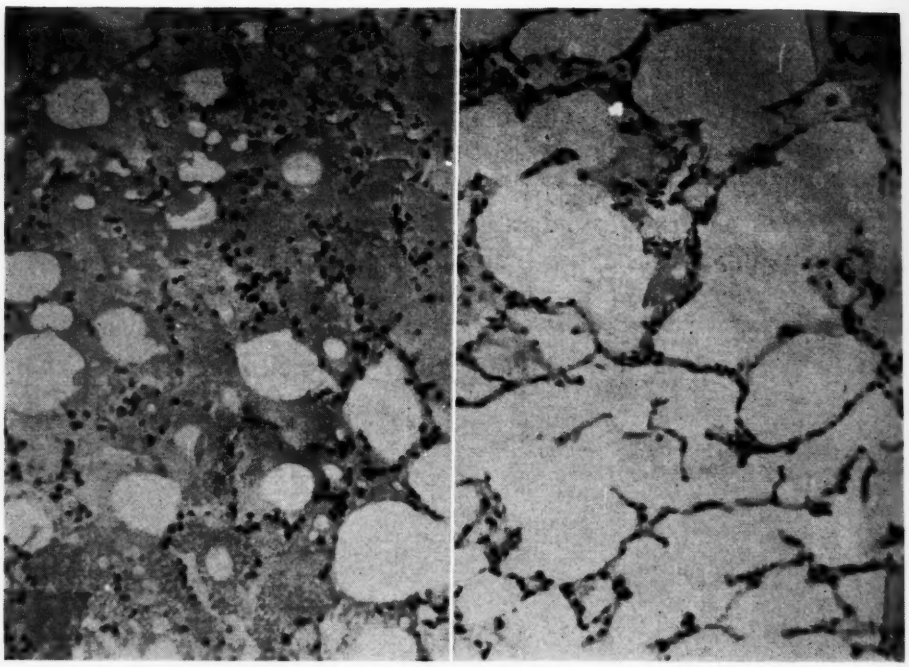


Fig. 3.—Left. Brain sensitization. Allergen: hydrolyzed autoserum. Sensitizing injection: 0.2 c.c. Time interval: 18 days. Shock injection: 2 c.c. Rabbit killed 36 hours after shock injection. Brain edema. The lungs were normal.

Right. Control animal received only sensitizing injection of same antigen. No shock injection. Killed 20 days later. Normal brain. The lungs were normal.



Right lung. Left lung.

Fig. 4.—Lung sensitization. Allergen: hydrolyzed autoserum. Sensitizing injection: 0.5 c.c. Time interval: 6 weeks. Shock injection: 2 c.c. Rabbit killed 48 hours after shock injection. Alveolar edema of right lung. Slight reaction of the opposite lung.

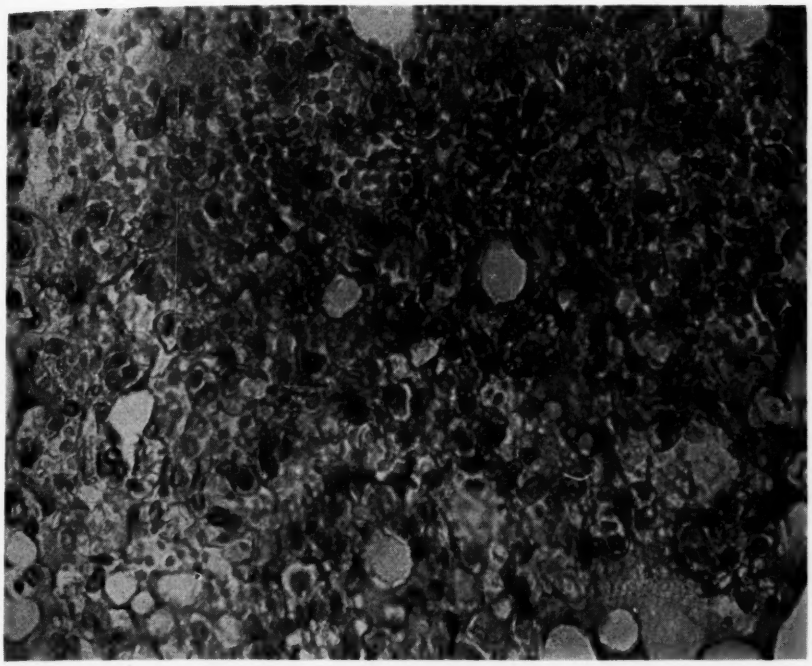


Fig. 5.—Lung sensitization. Allergen: homologous liver. Sensitizing injection: 0.5 c.c. Time interval: 6 weeks. Shock injection: 2 c.c. Rabbit killed 24 hours after shock injection. Alveolar edema, exudation of fibrin and red blood cells, infiltration by isolated large mononuclear cells (phagocytic), atelectasis, and destruction of alveolar pattern.

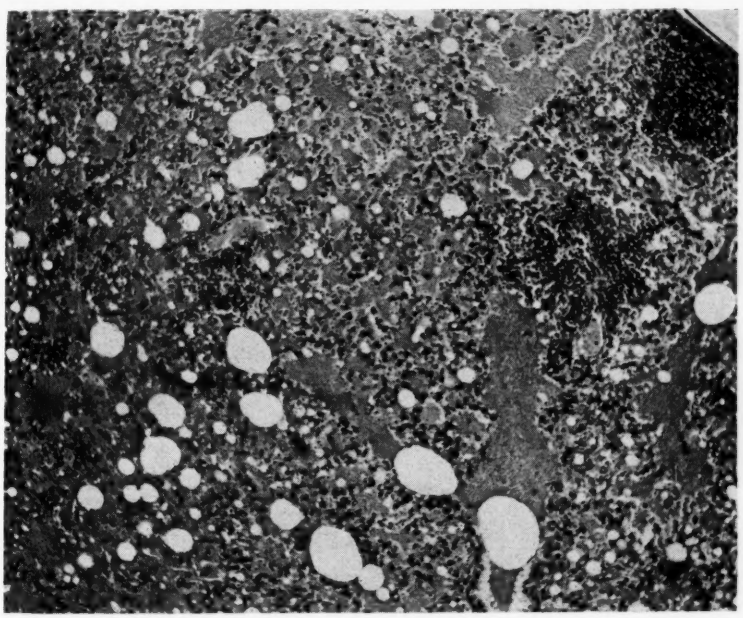


Fig. 6.—Lung sensitization. Allergen: homologous liver. Sensitizing injection: 1 c.c. Time interval: 25 days. Shock injection: 2 c.c. Rabbit killed 72 hours after shock injection. Alveolar edema, areas of necrosis of alveolar septa, and focal periarteriolar infiltration of eosinophiles.

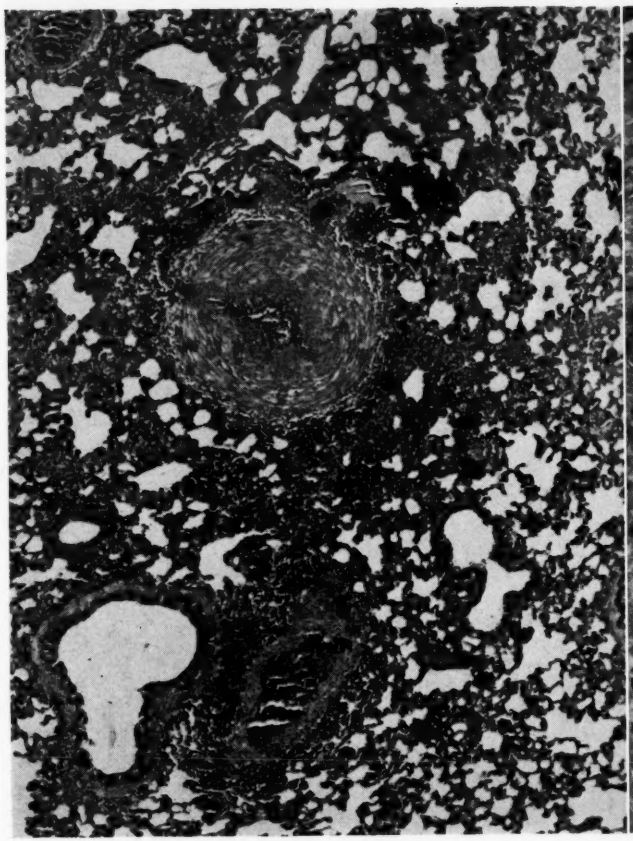


Fig. 7.

Fig. 7, *A*.

Fig. 7.—Lung sensitization. Allergen: autogenous skin. Sensitizing injection: 0.5 c.c. Time interval: 16 days. Shock injection: 1.5 c.c. Rabbit killed 48 hours after shock injection. Dense infiltration of eosinophiles and small and large mononuclears around artery and vein.

Fig. 7, *A*.—Artery of Fig. 7. High power. Endothelial proliferation, swelling, and desquamation.

24 hours it had a lacunar appearance, together with an infiltration by occasional eosinophilic cells*; after 48 hours, it often had a fibrinoid appearance. The media was the site of an edema which was maximum at 24 to 48 hours and decreased after 48 hours; in the more severe reactions this was associated with partial destruction of the media. In the smaller arterioles, especially after 48 hours, hyalinization and necrosis of the media, together with infiltration by eosinophilic cells and mononuclears, were noted in some cases.

Adventitia and periarterial region.—The earliest change, seen within the first hour, was an exudate of serum in the periadventitia and adjacent alveoli. Animals killed at 24 hours showed an extensive periarterial edema containing a variable number of cells. Animals killed after 48 hours showed in general a less extensive edema and a greater number of cells; the cellular infiltration consisted mainly of eosinophilic cells and small round cells; one or the other of these types of cells predominated in different animals; large mononuclear cells were often present and occasionally a few polymorphonuclears were to be seen.

Veins.—The veins were in general less frequently involved than the arteries. When they were, they showed the same endothelial reaction and perivascular edema and infiltration as in the arteries. During the first 48 hours marked congestion was noted. In some animals venous thrombi were seen. In a few cases there was a necrosis of the wall of the vein with infiltration by mononuclear and polymorphonuclear cells.

The perivascular lymphatics sometimes showed an increase in their lymphocyte content.

Alveoli.—The main pathological features were a thickening of the alveolar septa, alveolar edema, and atelectasis.

The thickened alveolar septa showed congestion (which was most marked during the first 24 hours), capillary thrombi in some animals, and proliferation and desquamation of septal cells.

The alveolar edema appeared as early as the first hour and decreased somewhat after 48 hours. It was variable in composition. In some instances there was a massive edema, composed of serum, which left the alveolar structure relatively intact; in other animals there was an edema composed of serum, red blood cells, and fibrin, often destroying the alveolar pattern. As a rule there was little cellular infiltration; when present, it consisted of isolated large mononuclear cells. In the later days, however, foci of eosinophilic cells from a periarteriolar infiltration extended into the adjacent alveoli and an increasing number of alveolar macrophages were found. In a few instances in which the edema was absent, an interstitial infiltration of mononuclear cells occurred.

Bronchi.—Serum and red blood cells were present in the lumen of many bronchi. When a vessel in the wall of a bronchus or bronchiole was the site of a marked perivascular infiltration, the invading cells often infiltrated the wall of the bronchus, but the bronchial epithelium remained intact.

*These cells had a bilobed nucleus and large eosinophilic granules and by comparison with other granulocytes appeared to be true eosinophiles.

and large mononuclears around artery and vein.
Fig. 7. A.—Artery of Fig. 7. High power. Endothelial proliferation, swelling, and desquamation.

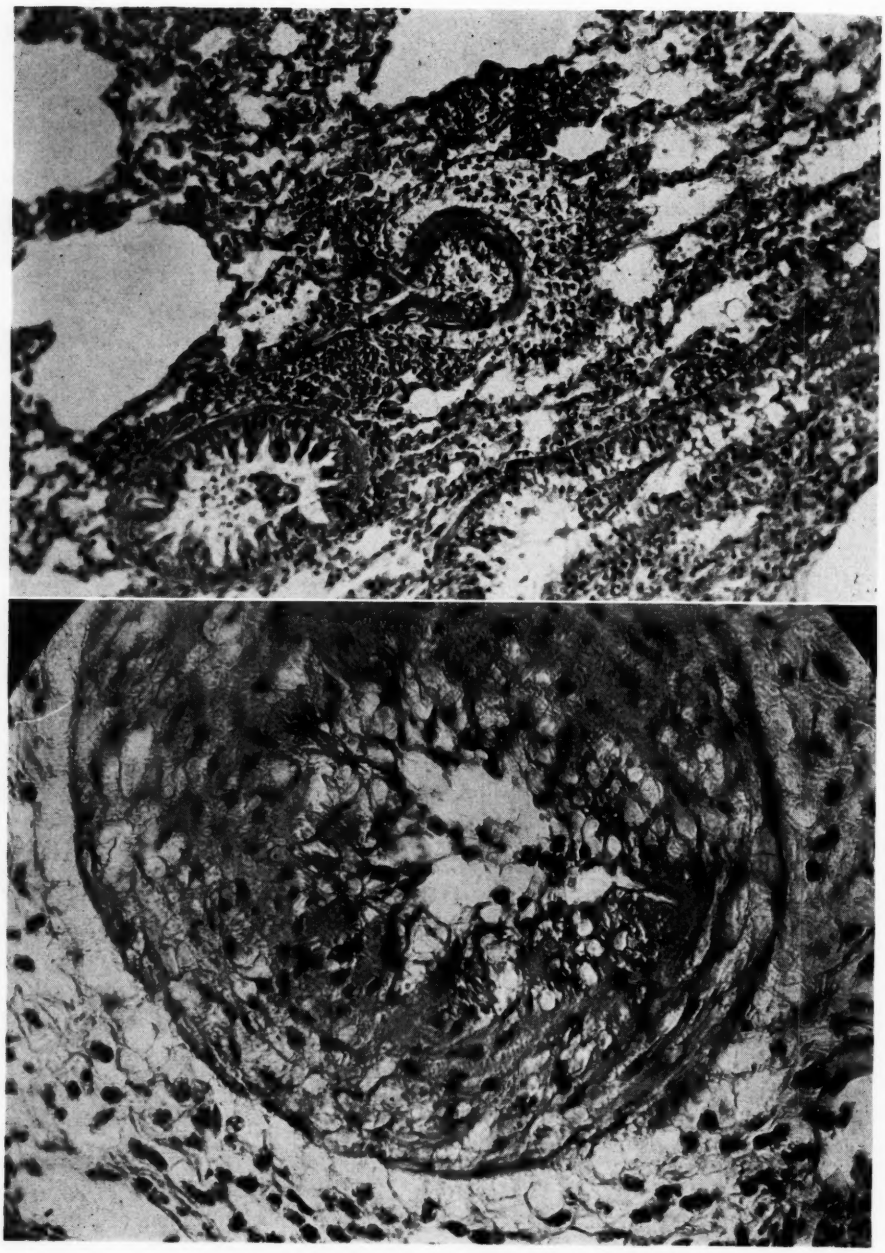


Fig. 8.

Fig. 8, *A*.

Fig. 8.—Lung sensitization. Allergen: homologous liver. Sensitizing injection: 0.5 c.c. Time interval: 6 weeks. Shock injection: 2 c.c. Rabbit killed 24 hours after shock injection. Endothelial swelling, proliferation, and desquamation; lacunar appearance of intima; moderate edema of media; perivascular infiltration of eosinophiles. High power.

Fig. 8, *A*.—Same rabbit as shown in Fig. 8. Endothelial reaction and perivascular infiltration in a swollen arteriole.

Pleura.—In some animals there was an exudative reaction of the pleura with serum, red blood cells, and often fibrin. There was little cellular infiltration. The lymphocyte content of the pleural lymphatics was often increased.

Controls:

The various types of controls showed, in general, normal lungs. The following features were however present in some of them: Atelectasis, with a thickening of the alveolar septa, without changes in their structure, was seen very frequently and was probably an artefact of fixation. In a few cases, there was slight alveolar congestion and patches of alveolar edema. In the arteries, slight desquamation of the endothelium was found at times, as well as thickening of the wall which appeared to be an artefact and could not be confused with the swelling due to endarterial edema in the experimental animals. Occasionally, alveolar macrophages, old pleural thickening, and lesions due to parasites were found.

COMMENT

The experiments herein reported indicate the development of specific pulmonary lesions in the rabbit by the use of the above described autogenous antigens. In an animal which had previously received a local injection of a given antigen, the reintroduction of the same antigen in intravenous injection after a suitable time interval, resulted in a lesion at the site of the primary local injection. Neither the intravenous nor the local injection alone caused such lesions. This is regarded as an anaphylactic reaction in the organ due to a locally induced hypersensitivity.

The pneumonitis obtained represents an allergic vascular reaction of the lung involving relatively large vessels as well as arterioles, venules, and alveolar capillaries. The lesions resemble those obtained in the rabbit's lung with other methods of sensitization and with exogenous antigens by Gregory and Rich,[35] Fried,[36, 37] Cannon, Walsh, and Marshall[38] and Herbut and Kinsey,[39] although they do not show the transitory alveolar infiltration with granulocytes described by the latter three authors. They also resemble in many respects the lesions observed in early stages of rheumatic pneumonia by Epstein and Greenspan[40] and, in certain respects, those observed by Harkavy[41] in eosinophilic pneumonitis.

The immunological features of the locally sensitized organ have been studied in the eye and with foreign antigens by Seegal and Seegal,[42-44] Seegal, Seegal, and Khorazo,[45] and Thompson, Gallardo, and Khorazo.[46] Thompson, Gallardo, and Khorazo showed that there was primarily a local formation of antibodies in the corneal stroma and that circulating antibodies appeared only secondarily. There is no reason to believe that the sequence of events is different when the lung is the sensitized organ, or when an endogenous, rather than an exogenous, antigen is used. Local organ hypersensitivity may therefore be interpreted as corresponding to a local formation of fixed tissue antibodies in the sensitized organ (local immunity).

Lesions in the contralateral organ were observed in some animals sensitized in the lung and in some animals sensitized in the eye. There is no satisfactory explanation of the mechanism of this phenomenon. One may suppose that some

Fig. 8, *A*.—Same rabbit as shown in Fig. 8. Endothelial reaction and periarterial infiltration in a swollen arteriole.

of the antigen injected in one lung or in one eye may have reached the contralateral organ by retrograde lymphatic flow in concentration sufficient to sensitize. This is, however, purely hypothetical.

The experiments showed that hydrolyzed autoserum, homologous liver, autogenous skin, and autogenous urine are capable of producing an allergic pneumonitis in the animal which served as their source of origin or in an animal of the same species. We are planning to continue these studies and investigate the specificity of the autogenous material used.

The first allergen was obtained by a partial hydrolysis of autogenous serum. It may be inferred that the autosensitizing power was due to a change in specificity caused by hydrolysis. It has not yet been ascertained at which stages of hydrolysis the material acquires and then loses its autosensitizing power. It is known that changes in the specificity of proteins can be brought about by denaturation (MacPherson and Heidelberger[47]), mild hydrolysis (Landsteiner[48]) or even hydrolysis to proteoses (Stull and Hampton[49]). It may be recalled in this respect that Uwazumi[50] found that when rabbit serum is heated to 80° C., it acquires both a different specificity and the power to elicit the formation of autoantibodies.

The sensitization of the lung with saline extracts of other organs (skin, liver) shows that a normal body constituent can sensitize an organ in the same animal or an animal of the same species when placed in an abnormal environment. The role of autolysis in enhancing the autoantigenicity in this case is difficult to evaluate.

The allergens present in autogenous urine may have consisted of proteins in amounts too minute to be detected by the usual methods, or of other simpler substances which could play the rôle of haptens, capable of combining with the local tissue proteins of the sensitized organ. Being excretory products, they behaved as foreign allergens when they were reintroduced in the organism and placed in an abnormal environment.

These experiments may have certain clinical implications. The results obtained with hydrolyzed autoserum indicate that denaturation and partial hydrolysis of autogenous proteins may give rise to autogenous antigens. This further suggests that autolysis could play the same role in man. The experiments with homologous liver and autogenous skin suggest that cellular destruction or necrosis in man can also give rise to autogenous antigens.

In other experimental work,[9, 18-31] the autoantigens used to produce lesions had been activated by the addition of foreign antigens, usually bacterias. While these experiments give a possible explanation for the presence of autogenous antigens in various infectious diseases, they do not give one applicable to those diseases in which there is no evidence of an infectious process. Autolysis and cellular destruction due to various causes such as nonbacterial toxic agents, metabolic and vascular diseases, as well as infectious agents, may now be conceived as possible sources of autogenous antigens in man.

Indeed, there is some clinical evidence to show that the presence of autoantigens is not limited to diseases in which infectious agents play a role. Autoantibodies against liver have been found not only in infectious hepatitis, but also

in toxic hepatitis, acute yellow atrophy, obstructive jaundice, and other diseases (Eaton, Murphy, and Hanford,[51] Bjørneboe and Krog[52]). It has been possible to desensitize certain patients who showed allergic phenomena following several applications of radium by repeated exposures to small doses of radium; these patients were then able to sustain the large doses without reaction. Hypothesis based on the role of autogenous antigens have been advanced for such diseases as multiple sclerosis (Rivers, Sprunt, and Berry[19]) and peptic ulcer (Jahiel[53]) in which there is, at the present time, no evidence of an infectious process. In glomerulonephritis and rheumatic fever the multiplicity of the lesions or symptoms leads one to suspect that more than one antigen or mechanism is operating.

This hypothesis may find an application in the field of tumors. Hodgkin's disease in which there is often a considerable tissue destruction, sometimes shows pathological features which suggest an allergic process, such as the presence of eosinophiles, periarterial phenomena in the spleen, and in certain cases, amyloid degeneration (Feldman[54]). It would be interesting to find out if autoantigens are liberated and what role they could play in the evolution of the disease.

In connection with the excretory product, urine, used in our experiments, it may be recalled that other abnormally reabsorbed excretory products, such as menstrual blood (Jahiel,[55] Salen[56]) have been found to cause certain allergic manifestations in women. In the case of the menstrual allergies, it was possible to desensitize patients with their own menstrual discharge.

SUMMARY

1. An allergic pneumonitis was obtained in the rabbit by using various autogenous materials as allergens.

2. The autogenous allergens used were: (1) partly hydrolyzed autogenous serum; (2) saline extracts of autogenous skin and homologous liver; and (3) an excretory product, autogenous urine. A complementary study was done with exogenous allergens.

3. A local sensitization of the lung was achieved by a single intrapulmonary injection of the allergen under investigation. After a suitable time interval, the shock injection of the same allergen was given intravenously and an allergic pneumonitis developed in the sensitized lung.

4. The pneumonitis was characterized by alveolar lesions (congestion, edema) and vascular lesions (endothelial reaction, endarterial, and periarterial edema, periarterial infiltration with eosinophilic cells and small and large mononuclears).

5. The immunological mechanism involved, the factors granting antigenicity to the autogenous substance used in these experiments and the possible events capable of leading to the release of similar allergens in man are discussed.

6. These experiments suggest that partial hydrolysis of autogenous proteins, tissue destruction and liberation of endocellular material, and reabsorption of an excretory product can, under certain conditions, give rise to autogenous allergens and provoke pathological changes in a previously sensitized tissue.

REFERENCES

1. Metalnikoff, S.: Etudes sur la spermotoxine, Ann. Inst. Pasteur. **14:** 577, 1900.
2. Woods, A. C.: Immune Reactions Following Injuries to the Uveal Tract, J. A. M. A. **77:** 1317, 1921.
3. Hektoen, L., and Schulhof, K.: Further Observations on Lens Precipitins; Antigenic Properties of α and β Cristallins, J. Inf. Dis. **34:** 433, 1924.
4. Krusius, cited by Landsteiner, K.: The Specificity of Serological Reactions, Cambridge, 1945, Harvard University Press, p. 61.
5. Lewis, J. H.: Isoantigenic Properties of Casein, J. Infect. Dis. **55:** 168, 1934.
6. Bruynoghe, G.: Le pouvoir isoantigenique de l'ovalbumine, Compt. rend. Soc. de biol. **118:** 1260, 1935.
7. Schwentker, F. F., and Rivers, T. M.: Antibody Response of Rabbits to Injections of Emulsions and Extracts of Homologous Brain, J. Exper. Med. **60:** 559, 1934.
8. Fischer, O., cited by Landsteiner, K.: The Specificity of Serological Reactions, Cambridge, 1945, Harvard University Press.
9. Burky, E. L.: Production in Rabbits of Hypersensitive Reactions to Lens, Rabbit Muscle and Low Ragweed Extracts by Action of Staphylococcus Toxin, J. ALLERGY **5:** 466, 1934.
10. Hecht, R., Sulzberger, M., and Weill, H.: Studies in Sensitization to Skin; Production of Antibodies to Skin by Means of Synergetic Action of Homologous Skin Antigen and Staphylococcus Toxin, J. Exper. Med. **78:** 59, 1943.
11. Lewis, J. H.: Immunologic Specificity of Brain Tissue, J. Immunol. **24:** 193, 1933.
12. Kopeloff, L. M., and Kopeloff, N.: Production of Antibrain Antibodies in Monkeys, J. Immunol. **48:** 297, 1944.
13. Schwentker, F., and Comploier, F. C.: Production of Kidney Antibodies by Injection of Homologous Kidney Plus Bacterial Toxins, J. Exper. Med. **70:** 223, 1939.
14. Cavelti, P., and Cavelti, E.: Studies on Pathogenesis of Glomerulonephritis; Production of Autoantibodies to Kidney in Experimental Animals, Arch. Path. **39:** 148, 1945.
15. Cavelti, P.: Studies on the Pathogenesis of Rheumatic Fever. Experimental Production of Autoantibodies to Heart, Skeletal Muscle and Connective Tissue, Arch. Path. **44:** 1, 1947.
16. Miyakava, cited by Urbach, E., and Gottlieb, P. M.: Allergy, ed. 2, New York, 1946, Grune & Stratton, Inc., p. 121.
17. Kidd, J. C., and Friedwald, W. F.: Natural Antibody That Reacts in Vitro With Sedimentable Constituent of Normal Tissue Cells; Demonstration of Phenomenon. Natural Antibody That Reacts in Vitro With Sedimentable Constituent of Normal Cells; Specificity of the Phenomenon; General Discussion, J. Exper. Med. **76:** 543, 557, 1942.
18. Rivers, T. M., Sprunt, D. H., and Berry, G. P.: Observations on Attempts to Produce Acute Disseminated Encephalomyelitis in Monkeys, J. Exper. Med. **58:** 39, 1933.
19. Rivers, T. M., and Schwentker, F. F.: Encephalomyelitis Accompanied by Myelin Destruction Experimentally Produced in Monkeys, J. Exper. Med. **61:** 689, 1935.
20. Ferraro, A., and Jervis, G.: Experimental Disseminated Encephalopathy in Monkey, Arch. Neurol. & Psychiat. **43:** 195, 1940.
21. Morgan, I. M.: Allergic Encephalomyelitis in Monkeys in Response to Injection of Normal Monkey Nervous Tissue, J. Exper. Med. **85:** 131, 1947.
22. Kabat, E. A., Wolf, A., and Bezer, A. E.: Rapid Production of Acute Disseminated Encephalomyelitis in Rhesus Monkeys by Injection of Heterologous and Homologous Brain Tissue With Adjuvants, J. Exper. Med. **85:** 117, 1947.
23. Wolf, A., Kabat, E. A., and Bezer, A. E.: Pathology of an Acute Disseminated Encephalomyelitis Produced Experimentally in Rhesus Monkey and Its Resemblance to Human Demyelinating Disease, J. Neuropath. & Exper. Neurol. **6:** 133, 1947.
24. Kabat, E. A., Wolf, A., and Bezer, A. E.: Studies on Acute Disseminated Encephalomyelitis Produced Experimentally in Rhesus Monkey. III. J. Exper. Med. **88:** 417, 1948.

24a. Kabat, E. A., Wolf, A., and Bezer, A. E.: Studies on Acute Disseminated Encephalomyelitis Produced Experimentally on Rhesus Monkeys. Disseminated Encephalomyelitis Produced in Monkeys With Their Own Brain Tissue. IV. J. Exper. Med. **89:** 395, 1949.

25. Morrison, L. R.: Disseminated Encephalomyelitis Experimentally Produced by Use of Homologous Antigen, Arch. Neurol. & Psychiat. **58:** 391, 1947.
26. Freund, J., Stern, E. R., and Pisani, T. M.: Isoallergic Encephalomyelitis and Radiculitis in Guinea Pigs After One Injection of Brain and Mycobacteria in Water-in-oil Emulsion, J. Immunol. **57:** 179, 1947.
27. Kopeloff, L. M., and Kopeloff, N.: Neurologic Manifestations in Laboratory Animals Produced by Organ (Adjuvant) Emulsions, J. Immunol. **57:** 229, 1947.
28. Ferraro, A., and Cazullo, C. L.: Chronic Experimental Encephalomyelitis in Monkeys, J. Neuropath. & Exper. Neurol. **7:** 3, 1948.

28a. Ferraro, A., and Cazullo, C. L.: Prevention of Experimental Allergic Encephalomyelitis in Guinea Pigs, J. Neuropath. & Exper. Neurol. **8**: 61, 1949.

28b. Cazullo, C. L., and Ferraro, A.: Production of Experimental Allergic Encephalomyelitis in Guinea Pigs Via the Intraperitoneal Route, J. Neuropath. & Exper. Neurol. **8**: 70, 1949.

28c. Lumsden, C. E.: Experimental Allergic Encephalomyelitis, Brain **72**: 198, 1949.

29. Jarvis, C., and Koprowsky, H.: Experimental Allergic Encephalomyelitis, J. Neuropath. & Exper. Neurol. **7**: 309, 1948.

30. Cavelti, P. A., and Cavelti, E. S.: Studies on Pathogenesis of Glomerulonephritis; Production of Glomerulonephritis in Rats by Means of Autoantibodies to Kidney. Studies on the Pathogenesis of Glomerulonephritis; Clinical and Pathological Aspects of Experimental Glomerulonephritis Produced in Rats by Means of Autoantibodies to Kidneys, Arch. Path. **40**: 158, 163, 1945.

31. Cavelti, P.: Studies on Pathogenesis of Rheumatic Fever; Cardiac Lesions Produced in Rats by Means of Autoantibodies to Heart and Connective Tissue, Arch. Path. **44**: 13, 1947.

32. Jahiel, R.: Pneumopathies expérimentales par sensibilisation protéinique, Rev. de la tuberc. **5**: 485, 1939.

33. Gosset, A., Jahiel, R., and Delaunay, S.: Etude du cycle évolutif d'une maladie pneumonique obtenue chez le lapin par anaphylaxie locale en partant de proteines endogénes modifiées, Compt. rend. Acad. d. sc. **208**: 140, 1939.

34. Jahiel, R., Rosen, M., and Morel, R.: Anaphylaxie pulmonaire locale obtenue chez le lapin à partir de sa propre urine, Compt. rend. Soc. de biol. **130**: 1005, 1939.

35. Gregory, J. E., and Rich, A. R.: Experimental Production of Anaphylactic Pulmonary Lesions With Basic Characteristics of Rheumatic Pneumonitis, Bull. Johns Hopkins Hosp. **78**: 1, 1946.

36. Fried, B. M.: Allergic Lobar Pneumonia; Experimental Study, J. Exper. Med. **57**: 111, 1933.

37. Fried, B. M.: Allergic Inflammation of Lungs; Pathogenesis of Lobar Pneumonia, Am. J. Path. **18**: 865, 1934.

38. Cannon, P. R., Walsh, T. E., and Marshall, C. E.: Acute Local Anaphylactic Inflammation of Lungs, Am. J. Path. **17**: 777, 1941.

39. Herbut, P. A., and Kinsey, F. R.: Transitory Pulmonary Infiltration (Loeffler's Syndrome), Arch. Path. **41**: 489, 1946.

40. Epstein, E. L., and Greenspan, E. B.: Rheumatic Pneumonia, Arch. Int. Med. **68**: 1074, 1941.

41. Harkavy, J.: Vascular Allergy, J. ALLERGY **14**: 507, 1943.

42. Seegal, D., and Seegal, B. C.: Local Organ Hypersensitiveness; Experimental Production in Rabbit's Eye, Proc. Soc. Exper. Biol. & Med. **27**: 390, 1930.

43. Seegal, D., and Seegal, B. C.: Local Organ Hypersensitiveness; Further Observations on Its Experimental Production in the Rabbit Eye, J. Exper. Med. **54**: 249, 1931.

44. Seegal, B. C., and Seegal, D.: Local Organ Hypersensitiveness; Demonstration of Agglutinins in Tissues of Rabbit Eye After Immunization With Eb. Typhi Vaccine, Proc. Soc. Exp. Biol. & Med. **31**: 437, 1934.

45. Seegal, B. C., Seegal, D., and Khorazo, D.: Local Organ Hypersensitiveness; Fate of Antigen and Appearance of Antibodies During Development of Hypersensitiveness in Rabbit Eye, J. Immunol. **25**: 207-220, 1933.

46. Thompson, R., Gallardo, E., and Khorazo, D.: Precipitins in Ocular Tissues of Rabbits Generally and Locally Immunized With Crystalline Egg Albumin, Am. J. Ophth. **19**: 852, 1936.

47. MacPherson, C. F. C., and Heidelberger, M.: Preparation and Immunological Properties of Acid-Denaturated Egg Albumin, Proc. Soc. Exper. Biol. & Med. **43**: 646, 1940.

48. Landsteiner, K.: Specificity of Serological Reactions, Cambridge, 1945, Harvard University Press.

49. Stull, A., and Hampton, S. F.: Study on Antigenicity of Proteoses, J. Immunol. **41**: 143, 1941.

50. Uwazumi, cited by Landsteiner: Specificity of Serological Reactions, Cambridge, 1945, Harvard University Press, p. 44.

51. Eaton, M. D., Murphy, W. D., and Hanford, V. L.: Heterogenetic Antibodies in Infectious Hepatitis, J. Exper. Med. **79**: 539, 1944.

52. Bjørneboe, M., and Krag, P.: Studies on Complement Fixation in Hepatitis With Liver Extract as Antigen, Acta path. et microbiol. Scandinav. **24**: 352, 1947.

53. Jahiel, R.: Ulcére gastro-duodenal et mémoire cellulaire, Arch. d. mal. de l'app. digestif. **28**: 627, 1938.

54. Feldman, D.: Oral communication.

55. Jahiel, R.: Sur des etats de sensibilisation hépatique en rapport avec la fonction ovarienne normale, Arch. d. mal. de l'app. digestif. **24**: 9, 1934.

56. Salen, E. B.: Spezifishes Allergen aus Menstruationsblut. Ein Beitrag zur Frage der endogen Ursachen der allergishen Symptomencomplexe. Acta med. Scandinav. **59** (Suppl.): 494-535, 1934.

HISTAMINE INDUCED CARDIOVASCULAR LESIONS*†

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IN investigations on serous inflammation Eppinger, Kaunitz, and Popper[1] produced endocardial and myocardial lesions in dogs through the administration of allylformiat and of allylformiat and histamine. These lesions were thought by them to be similar to those of rheumatic heart disease. Dammin and Bukantz,[2] however, were not able to produce such lesions in rabbits through the intravenous administration of histamine.

In view of the theoretical importance of this question we again investigated the effect of histamine administration on the organs of rabbits, guinea pigs, and rats.

EXPERIMENTAL

A. Rabbits

Twenty-one young male rabbits weighing between 1700 and 2700 grams were given intraperitoneal injections of histamine acid phosphate (Burroughs Wellcome & Co., Inc.). The individual doses were dissolved in 10 ml. of distilled water. The smaller animals received from 7.5 to 8 mg. of histamine acid phosphate per kilogram of body weight per injection. The larger animals from 7.5 to 10 mg. Injections were given on 5 consecutive days, and then after a pause of 72 hours, they were again given in the same manner. In some animals, periods of from 7 to 10 days were interspersed in which no injections were given. The animals were sacrificed from 1 to 11 days after the last injection. In four instances the animals died immediately after an injection (Rabbits 7, 8, 9, 15). Table I records the total amount of histamine acid phosphate administered to each rabbit, the duration of observation in days, the number of days which elapsed between the last injection, and the time the animal was sacrificed. The intensity of the observed tissue lesions are designated as follows: 0 = no change, + = slight changes, ++ = moderate changes, +++ = marked changes, and ++++ = severe damage.

The histological changes observed are progressive and are dependent upon the duration of administration and the amount of histamine given. The lesions will therefore be described in the chronological order of their development. Not all of the lesions are observed in every animal receiving equal amounts over the same period and there are unexplained individual variations in the severity of the changes. The various lesions are illustrated in the accompanying microphotographs. They will not be referred to in the body of the article.

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†Under a grant from the Salzer Cardiovascular Fund.

TABLE I. RABBITS

RABBIT NUMBER	TOTAL DOSE MG. HISTAMINE BASE	INDIVIDUAL DOSE MG. HISTAMINE BASE	TOTAL NUMBER OF INJECTIONS	DAYS OBSERVED	SACRIFICED DAYS AFTER LAST INJECTION	VALVES	MYOCARDIUM	VEINS	ARTERIES	LUNGS
12	1.0	1	1	1	1	+	0	0	0	0
6	4.0	4	1	3	2	++	0	0	0	0
1	5.7	1-2	5	6	2	+++	0	0	0	0
8	17.0	1-4	5	11	0	+++	++	0	0	0
4	21.0	4-5	5	24	14	++	0	0	0	0
9	21.0	1-5	5	11	0	+++	++	++	+	++
10	26.0	1-5	6	13	1	++++	++	++	+	+++
5	27.8	1-5	10	12	3	++	0	0	0	0
16	55.0	5	11	17	2	+	++	++	+	+++
14	80.0	5	16	25	4	++++	++++	++++	+++	+++
17	100.0	5	20	39	9	+++	++++	+++	+++	++
7	137.0	0.7-5	27	64	0	+++	+++	+++	++	++
19	145.0	5	29	48	1	+++	+++	++	++	+++
22	146.7	5-6.66	17	41	11	+	++	+	+	+
15	180.0	5-6.66	35	60	0	+++	++	+++	++	+++
11	183.7	1-8.30	31	60	3	+++	+++	++	+++	+++
13	221.3	1-8.30	34	63	2	+++	++++	++++	+++	+++
18	258.3	5-6.66	42	74	11	++	++	++	+	++
24	278.3	5-6.66	42	86	7	++	++	+	++	++
20	331.7	5-6.66	29	102	2	+++	++	++	+++	++
23	341.7	5-6.66	32	92	2	+++	+++	+++	+++	++++

Several of the animals died from histamine shock either after the first dose or after considerable amounts of histamine had been previously given. All of these showed degeneration of the liver parenchyma, the cell cytoplasm undergoing hydrophic degeneration. These liver lesions are not part of the picture of chronic histamine poisoning and will not be discussed further.

The characteristic lesion is an inflammation of the endothelium and the underlying connective tissue of the cardiovascular system. Secondary changes, particularly in the lung, occur as a result of the vascular lesions.

With amounts of less than 100 mg. of histamine, administered over a period of from 2 to 21 days, the dominant lesions occur in the heart valves. Only one valve of the heart may show changes or all of the valves may show damage. Only one leaflet of a given valve or all of the leaflets may be involved. The initial change is an edema, which is sometimes extreme in degree and which is located beneath the endothelium of the valve (Figs. 1 and 2). The edema may be diffuse and involve the entire leaflet to its base or may be localized and involve only a portion of the leaflet. With increasing dosage and administration over a longer period, inflammatory cells appear in the areas of edema and these on occasion form collections. At times mucoid degeneration occurs in the areas of edema. After approximately 300 mg. of histamine have been given, the edge of the valve leaflet may present knoblike projections in and about which inflammatory cells may be found. We have not observed any inflammatory reactions comparable to those seen in the valves of acute rheumatic fever in man. At a still later period, when about 400 mg. of histamine have been given, fat cells make

their appearance on the edge of the valves replacing the endothelium. The lesions in the valves do not increase in severity beyond that observed when about 500 mg. have been administered, even though the dosage be increased to over 1000 mg. (Figs. 3 and 4).

While the changes in the valves are occurring, small areas of edema appear in the valve ring. Accompanying the edema is an extensive inflammatory cell exudate and, with amounts of 300 to 400 mg., fibrosis of moderate degree is observed in the inflammatory zones. Subendocardial areas of edema are also observed in the wall of the ventricles, and, while in some areas this edema is marked, inflammatory cells are not present (Fig. 5).

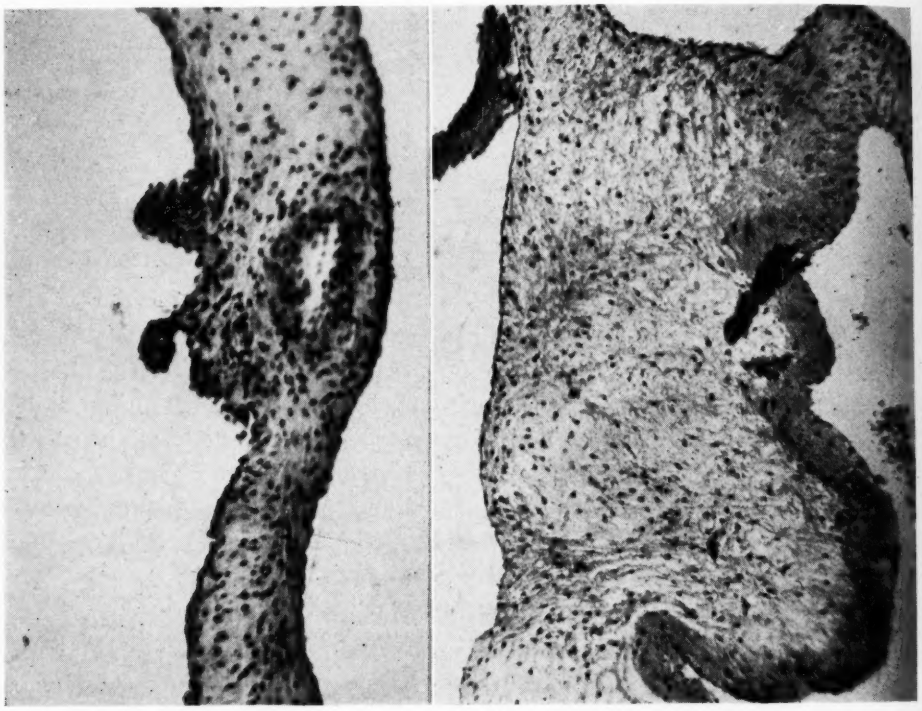

Fig. 1. Fig. 2.

Fig. 1.—Heart valve, rabbit, showing knoblike proliferations at edge. (8 mm. obj., 10 occ.)

Fig. 2.—Edema of heart valve, rabbit. (Magnification as in Fig. 1.)

With amounts of from 100 to 200 mg. of histamine, collections of inflammatory cells make their appearance in various parts of the myocardium. These collections are located about the smaller blood vessels, most often veins. The fact that Miller[3] has described similar lesions in normal rabbits raises some doubt as to their significance. We have observed, however, in and about such areas, small areas of hemorrhage and in several areas, edema and fragmentation of the muscle fibers with small areas of necrosis (Figs. 14 and 15). These lesions are distinct from the vascular lesions to be described later.

We have intentionally refrained from designating the inflammatory cells more specifically as to type or probable source of origin. They are large, and have large deeply staining nuclei with narrow rims of cytoplasm. Lipoid granules are frequently demonstrable in them.

The most striking lesion occurs in the vascular system where with histamine in amounts between 100 and 200 mg. the first of the vascular changes become evident in the lung. There is a perivascular inflammatory cell exudate first found about the smaller veins, progressing then to the larger veins, and finally to the largest veins of the lung. The lesion is focal and does not extend the length of the vesse[1]. At about the time the medium-sized veins in the lung show the perivascular infiltration the process starts in the vessels of the heart and in

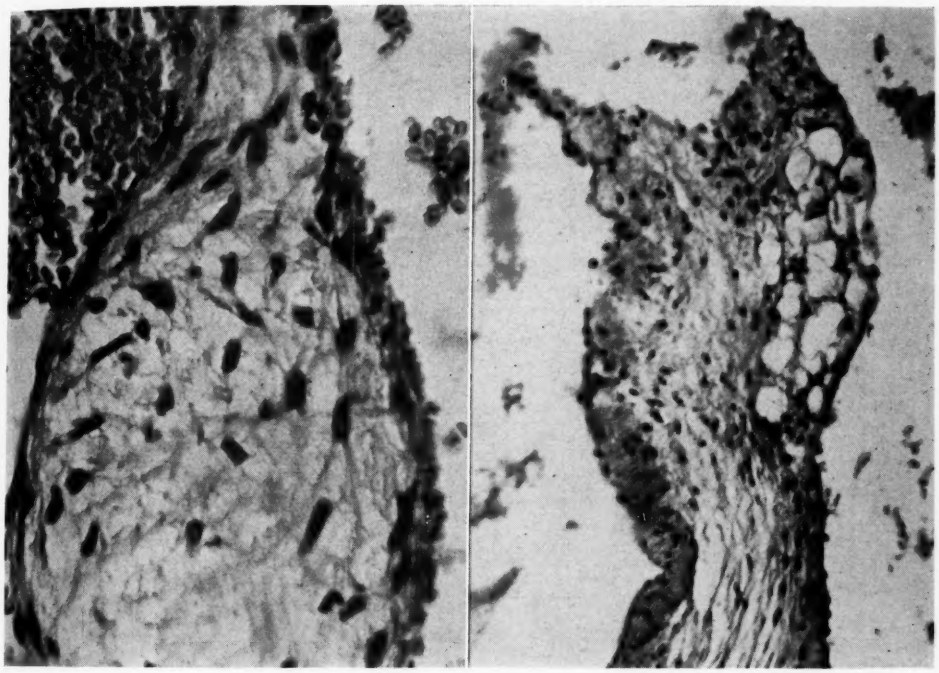

Fig. 3. Fig. 4.

Fig. 3.—Edema of heart valve, rat. (Magnification as in Fig. 1.)
Fig. 4.—Fat deposit in heart valve, rabbit. (Magnification as in Fig. 1.)

the small arteries of the lung. The reaction of the arteries starts in the small arterioles, it then extends to the larger arteries, and finally to the largest arteries of the organ (Figs. 6-10).

The development of the lesion is, we believe, unusual. The inflammatory cell exudate having formed, the wall of the vein becomes edematous. The inflammatory cells penetrate the edematous wall, possibly by amoeboid motion, and are found free in the vein lumen. Some of the inflammatory cells adhere to the endothelial lining of the vein which is also edematous. Once affixed to the wall of the vein the inflammatory cells send out a process which floats free in the vessel lumen until it comes in contact with a similar process arising from

an inflammatory cell affixed to another portion of the vessel wall. The cell processes unite. Other inflammatory cells attach themselves to this slender bridge and fill up the lumen of the vessel more or less completely so that on cross section the vein looks as if it were filled with areolar tissue.

The arterial changes differ slightly from those observed in the veins in that there is more perivascular edema but otherwise the process is the same and the artery lumen is finally more or less completely filled with what appears at first glance to be areolar tissue. At times the muscle fibers of the media are fragmented and the fibers of the adventitia are torn apart. In two of our animals the coronary arteries were involved in this degenerative change.

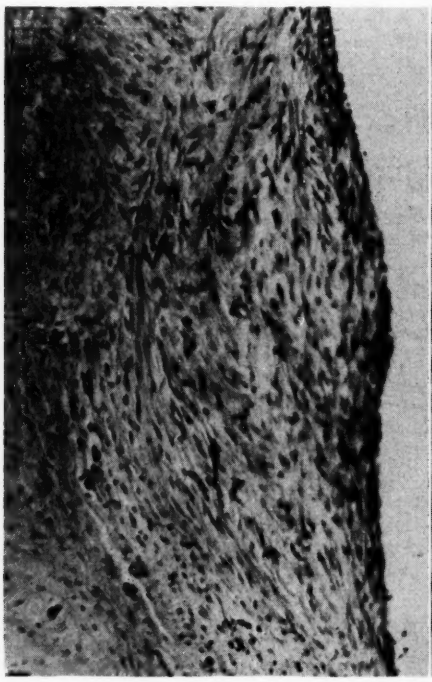

Fig. 5.

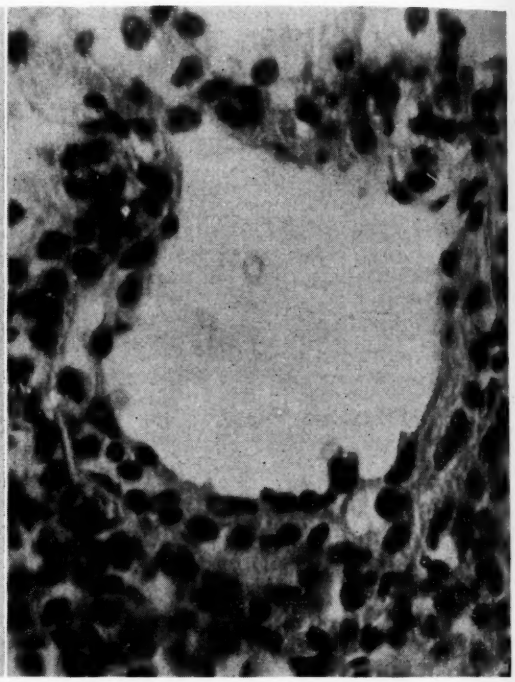

Fig. 6.

Fig. 5.—Area of proliferation in valve ring of rabbit heart. (8 mm. obj., occ. ×10)
Fig. 6.—Perivascular inflammatory cell exudate about vein, rabbit lung. Note edema of vessel wall and penetration of wall by inflammatory cell. (4 mm. obj., occ. ×10)

With two exceptions vascular lesions were not observed in any vessels other than those in the lungs and heart. In one animal there was a marked sclerosis of the arteries of the left lobe of the liver, while in the other there were scattered areas of sclerosis of the glomerular arteries of the kidney.

The changes secondary to the vascular lesions of the lung were extensive. In some areas, as the inflammatory cells penetrated the vein wall, minute hemorrhages occurred, sufficient blood escaping to fill 5 or 6 of the adjacent lung alveoli. In several animals these hemorrhages were so numerous as to suggest subpleural embolic petechiae. In the areas of hemorrhage the wall of the alveoli became edematous and infiltrated with inflammatory cells. The alveolar spaces became obliterated by compression. The areas of obliteration

of the alveoli were sharply demarcated from the adjacent noninvolved lung. In animals in which areas of this character were either numerous or extensive, the adjacent noninvolved lung showed the usual picture of emphysema with breaking down of the alveolar septa. With larger amounts of histamine, about 500 mg. and over, entire lobes of the lung were thus involved appearing in the gross as dark purple, almost black. In some areas the inflammatory reaction also involved the bronchi, peribronchial inflammatory cell exudate occurring with some edema and with the reaction extending into the bronchial wall.

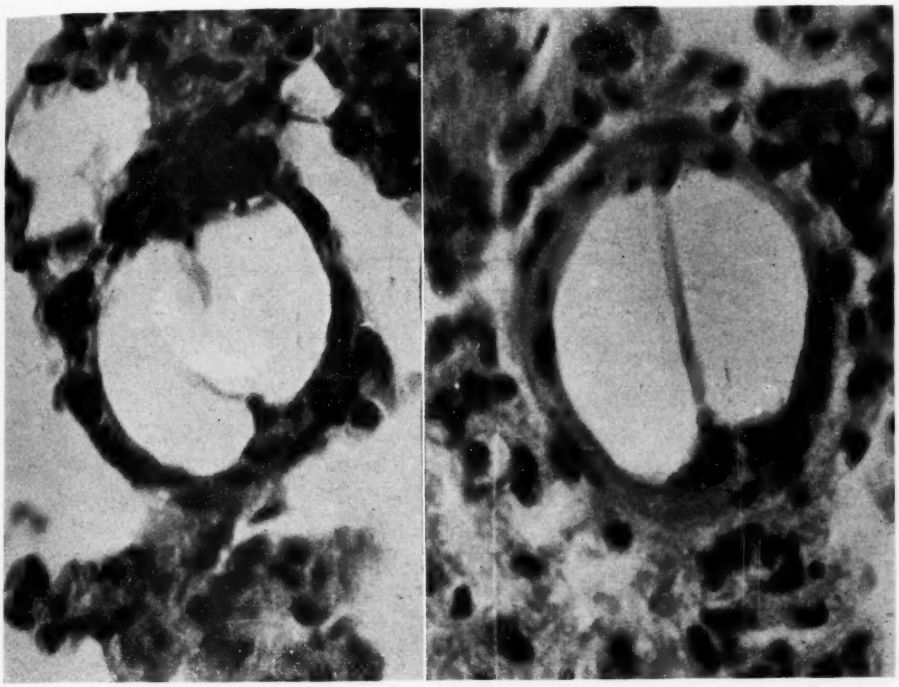

Fig. 7. Fig. 8.

Fig. 7.—Vein, rabbit lung, showing processes extending from inflammatory cells which have penetrated vessel wall. (4 mm. obj., occ. ×10)

Fig. 8.—Vein, rabbit lung, showing completion of bridge by inflammatory cells. (4 mm. obj., occ. ×10)

When the animals had been given more than 500 mg. of histamine, particularly when the dosage approached 1000 mg., the gross appearance of the lung was striking. In 2 animals one entire lung was dark purple and in another animal, which died of intercurrent disease, both lungs had this appearance. In these animals practically all the veins and arteries of the involved lung were filled with thrombi and there was a massive infarction of the organ which could not be explained by the proliferations within the vessel lumina. In the animal dying of intercurrent disease there were moderately extensive areas of liquefaction in both lobes of the left lung and there was bloody fluid in each pleural cavity (Figs. 16 and 17).

The secondary changes in the myocardium were slight. Minute areas of hemorrhage were occasionally observed about small veins. Completely blocked small vessels, usually arterioles, were not infrequent. These areas of blockade

were associated with narrow zones of necrosis which run parallel to the vessel. The hearts of animals showing advanced changes in the lungs were hypertrophied.

When the injections of histamine were stopped for a period of 10 days before the animal was sacrificed, recessive changes were evident. Thus, in an animal receiving a total of 600 mg. of histamine, most of the areas of inflammatory cell exudate had disappeared and only a few vessels with proliferation within the lumen were observed. The lung changes receded at least partially, although here and there areas of fibrosis in the lung parenchyma gave evidence of previous inflammatory reaction. The edema of the cardiac valve leaflets persisted but there was no deformity of the valve edges.

Apparently the lesions persist only as long as the histamine is given and do not progress beyond a certain phase after which the further administration of histamine is without effect on the tissues. Such changes as are observed after the administration of the histamine has been stopped are due to the secondary changes consequent upon interference with blood supply. No inflammatory changes were observed in tendon sheaths or in the joints.

B. Guinea Pigs

Twelve young male animals weighing between 225 and 250 grams were given 0.5 to 1.5 mg. of histamine acid phosphate per 100 grams of body weight by intraperitoneal injection. The histamine was dissolved in 5 ml. of distilled water. The injections were given on 5 consecutive days and then, after a pause of 72 hours, they were again given in the same manner. The animals were sacrificed from 24 hours to 5 days after the last injection. Two animals died immediately after injection (Guinea Pigs 5, 6).

The total amounts of histamine administered to each, the days of observation, etc., are recorded in Table II. The degree of tissue involvement is graded by the same symbols used in Table I.

TABLE II. GUINEA PIGS

GUINEA PIG NUMBER	TOTAL DOSE MG. HISTAMINE BASE	INDIVIDUAL DOSE MG. HISTAMINE BASE	NUMBER INJECTIONS	DAYS OBSERVED	SACRIFICED DAYS AFTER LAST INJECTION	VALVES	MYOCARDIUM	VEINS	ARTERIES	LUNGS
7	0.33	0.33	1	1	1	++	0	0	++	++
8	0.33	0.33	1	3	3	+	0	0	+	++
9	0.33	0.33	1	5	5	+	0	0	0	0
3	5.0	1.0	5	7	1	++	++	++	++++	++++
1	8.0	1.0	8	11	1	+++	++	+	+++	+++
2	10.0	0.5-1	15	14	1	+++	++	++	++++	++++
4	10.0	1.0	12	19	1	+++	++	+++	++++	+++
6	18.5	0.5-1	32	39	0	+++	+	++	++++	++++
10	20.0	0.5-1	24	25	2	+++	+	++	++++	+++
5	21.0	1.0	21	26	0	+++	++	++	++++	++++
11	31.0	1.0	31	37	2	+++	++	+	+++	++++
12	33.0	1.0	33	40	2	+++	++	++	+++	++++

The lesions observed in guinea pigs differ in some respects from those seen in the rabbit. The areas of edema in the cardiac valves and in the endocardium are comparable to those seen in the rabbit. The knoblike protuberances ob-

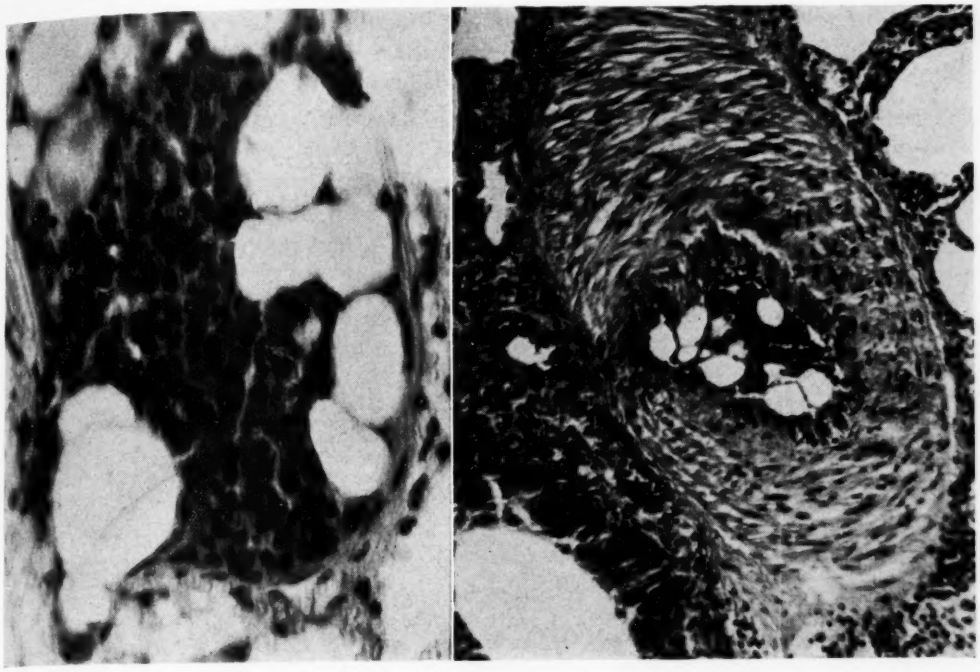

Fig. 9. Fig. 10.

Fig. 9.—Partial occlusion of large vein, by inflammatory cells, rabbit lung. (4 mm. obj., occ. ×10)
Fig. 10.—Same lesion as in Fig. 9 in artery, rabbit lung. (4 mm. obj., occ. ×10)

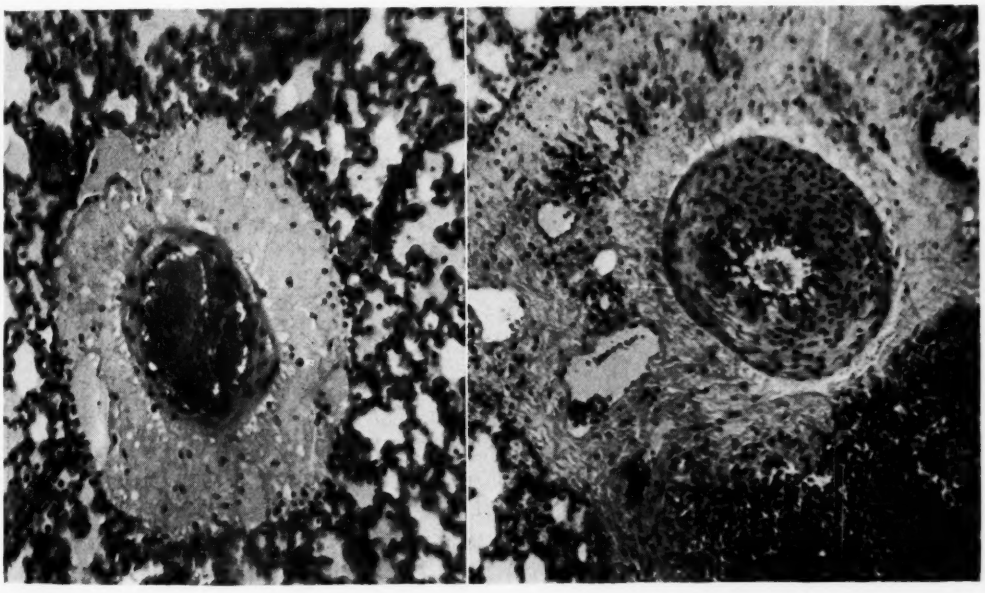

Fig. 11. Fig. 12.

Fig. 11.—Perivascular edema in lung, guinea pig. (8 mm. obj., occ. ×10)
Fig. 12.—Perivascular edema and focal inflammatory cell exudate in lung, guinea pig. (8 mm. obj., occ. ×10)

served on the valve edges show a greater inflammatory cell exudate than was observed in the rabbit. Areas of inflammatory cell collections in the myocardium are much less frequent than in the rabbit.

In the lungs the inflammatory cell infiltrations about the veins are both more numerous and more extensive than in the rabbit. There is, however, very little tendency of the inflammatory cells to penetrate the vein wall and in only one animal was an intralumen proliferation observed. There is, however, a very marked perivascular edema involving the arteries and the bronchial tree. Both the inflammatory cell exudate and the perivascular edema are in evidence after a total histamine dosage of 15 mg. The edema increases with increasing amounts of histamine, the perivascular inflammatory cell exudate does not (Figs. 11 and 12).

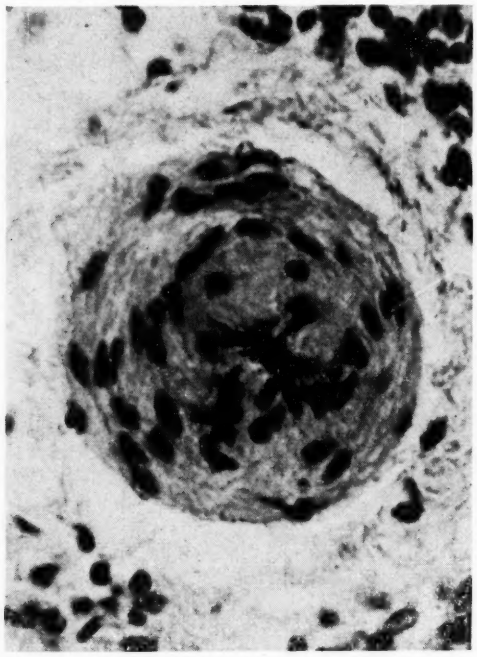

Fig. 13.—Comp'ete c'osure of artery in lung, guinea pig, slight perivascular edema and slight inflammatory perivascular exudate. (8 mm. obj., occ. ×10)

After approximately 25 mg. of histamine have been given the perivascular edema is very marked, the area involved not infrequently being twice the overall diameter of the vessel about which it occurs. As the edema increases, the fibers of the adventitia are torn apart. The diameter of the vessel wall increases due either to an apparent hypertrophy or swelling of the muscle fibers of the media until the lumen of the vessel is completely obliterated. The muscle fibers may then undergo hyaline degeneration leaving only a circular area of cell-poor connective tissue (Fig. 13).

Consequent upon the sclerosis of the smaller arteries, proliferative changes occur involving the lung parenchyma. The partitions between the alveolar spaces become thickened and the alveolus collapses. Inflammatory cells are not

frequently observed. The final picture resembles that seen in the rabbit. Grossly, the lung is spattered with dark-red or purplish irregular areas while on section there is increased resistance to the knife.

While these changes are going on in and about the arteries, the bronchial tree is also undergoing comparable changes. There is marked peribronchial edema with a localized collection of inflammatory cells at one point in the circumference of the bronchus. The edema tears apart the muscle fibers; the small bronchi which are devoid of cartilaginous support collapse, and the lumen becomes filled with desquamated or dislodged epithelial cells from the mucosa. We have not observed the massive infarction described in the rabbits.

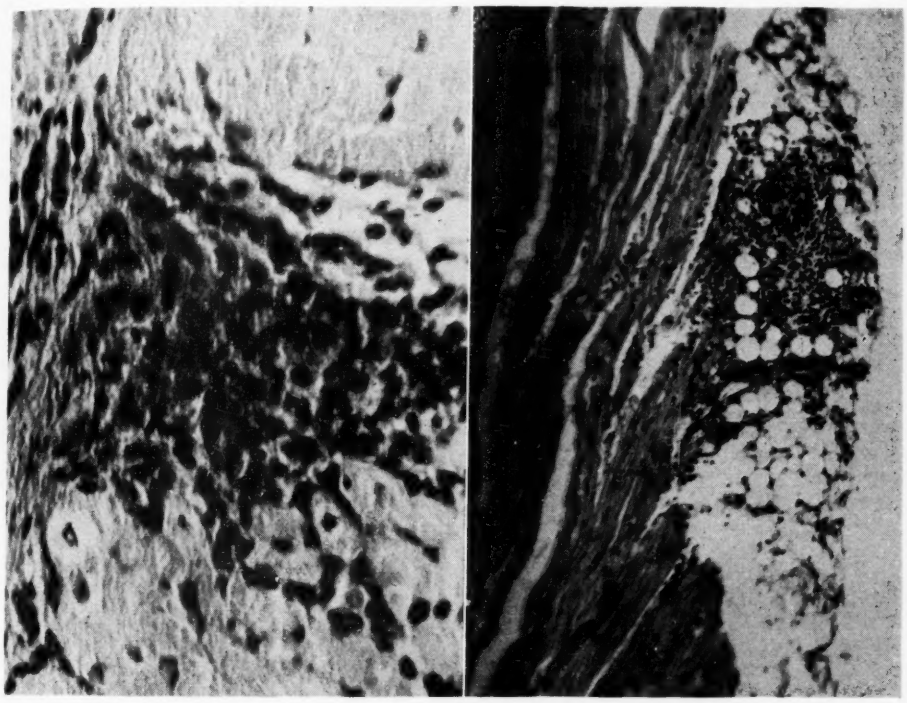

Fig. 14. Fig. 15.

Fig. 14.—Inflammatory cell exudate in myocardium of heart, rabbit. (8 mm. obj., occ. ×10)

Fig. 15.—Inflammatory cell exudate in visceral pericardium, rabbit. (8 mm. obj., occ. ×10)

In the heart of the guinea pig the same perivascular edema occurs but not to as marked a degree as in the lung. The thickening of the diameter of the arterial wall also occurs, but we did not observe any vessels of the vascular tree to show the complete closure observed in the lung.

In the guinea pig no lesions of the vascular system in any organ other than the lung and heart were observed. Six of the animals showed as an incidental finding ulcers of the stomach but the adjacent blood vessels in the region showed no lesions comparable to those observed in the lung.

In an additional series of 6 quinea pigs, not presented in the table, a single injection of 1.5 mg. of histamine acid phosphate was given. The animals were sacrificed in rotation at 24-hour intervals commencing one day after injection. In these the lesions of the heart valves became apparent within 24 hours after the injection and they were fairly well healed after 7 days.

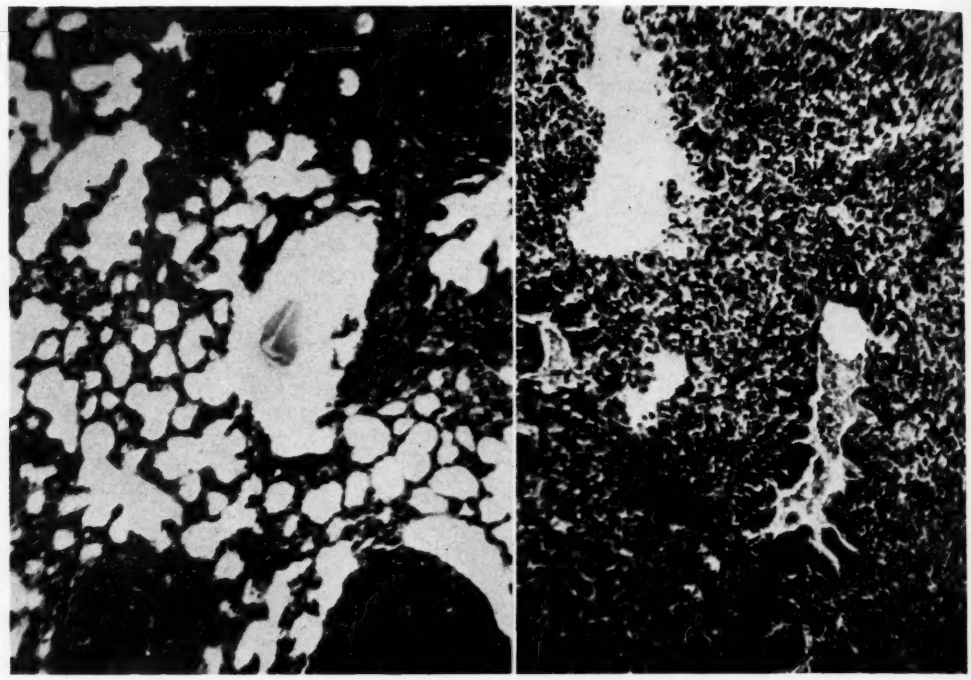

Fig. 16. Fig. 17.

Fig. 16.—Focal areas of intense inflammatory cell exudate, guinea pig lung. Intervening lung tissue fairly normal. (8 mm. obj., occ. ×10)

Fig. 17.—Extensive area of inflammatory reaction in guinea pig lung, bronchus still patent, occluded vessel at right of bronchus. (8 mm. obj., occ. ×10)

C. Rats

Six animals weighing between 150 and 175 grams were used. They were given 0.5 to 1.5 mg. of histamine acid phosphate per 100 grams of body weight dissolved in 5 c.c. of distilled water by intraperitoneal injection. The injections were given on 5 consecutive days and then, after a pause of 72 hours, they were again given in the same manner. The animals were sacrificed from 24 to 48 hours after the last injection. The total amounts of histamine administered in each animal, the days of observation, the degree of tissue involvement, etc., are recorded in Table III.

The lesions in rats were of the same character as those described for the rabbit and guinea pig. The edema of the valves was of moderate degree. The collections of inflammatory cells in the myocardium were much more infrequently observed than in the other two species but, since such collections of

inflammatory cells are not rare in normal rats, we have not attached great significance to them. Vascular changes in the heart aside from slight degrees of perivascular edema were not observed.

In the lungs the perivascular infiltration of inflammatory cells occurred with moderate frequency and in moderate degree. Edema of the vein walls accompanied by perivascular inflammatory cell exudate and perivascular edema of the arteries were also in evidence. Small areas of inflammatory reaction in the lung, with or without local infarction, were also present. As with the other species no lesions were found in organs other than the heart and lungs.

TABLE III. RATS

RAT NUMBER	TOTAL DOSE MG. HISTAMINE BASE	INDIVIDUAL DOSE MG. HISTAMINE BASE	NUMBER OF INJECTIONS	DAYS OBSERVED	SACRIFICED DAYS AFTER LAST INJECTION	VALVES	MYOCARDIUM	VEINS	ARTERIES	LUNGS
1	2.0	0.33	6	6	2	0	0	0	0	0
2	7.9	0.33	12	11	2	0	++	++	+++	+
3	12.9	0.66-1.0	17	19	2	+	+	+	++	0
4	19.0	0.66-1.0	23	26	1	+	+	0	++	+
5	25.0	0.66-1.0	28	35	2	+	0	0	0	0
6	25.0	0.66-1.0	28	35	2	+	0	0	0	0

Although the tissue changes observed in the rat were of the same character as those seen in the rabbit and guinea pig, they were of far less intensity. This is not surprising since the rat is very resistant to histamine poisoning.

DISCUSSION

In rabbits, guinea pigs, and rats, administration of histamine acid phosphate produced an inflammatory reaction in the vascular system of the heart and lungs and in the endo- and myocardium.

The lesions were sharply localized to the vascular tree of the heart and lung and to the endo- and myocardium. This rather puzzling phenomenon may possibly be explained on the following basis: The histamine was injected into the peritoneal cavity from which there is a very prompt resorption via either the lymphatics or the capillaries which carry it directly to the right heart. This transportation is rapid and during the passage the histamine is probably not appreciably altered. Passage through the arteries of the lung to the capillaries is also rapid. The first slowing of circulatory speed occurs in the capillaries of the lung. It is here that the first vascular lesions appear in the rabbit. From the venous radicals of the lung the histamine is carried to the left heart and then into the peripheral circulation where a rapid destruction of the histamine occurs.

Up to a certain point increasing amounts of histamine intensify the extent and degree of tissue damage. Having reached the point of maximum reaction, additional amounts of histamine exert no further effect. This is very likely due to induced tolerance to histamine. That such tolerance can be acquired through the repeated administration of histamine has been demonstrated by various

authors.[4] In some instances we have observed reparative changes in rabbits which were receiving large amounts of histamine. Katzenstein[5] reports the same observation in dogs.

SUMMARY

1. Data have been presented showing that the intraperitoneal administration of histamine acid phosphate in rabbits, guinea pigs, and rats produces an inflammatory reaction in the vascular system of the lung and the heart and in the endo- and myocardium.

2. Certain lesions are identical both in location and type in all 3 species, such as edema of the endocardium, inflammatory cell collections in the myocardium, edema of the heart valves, perivascular edema, and inflammatory cell exudate about the blood vessels.

3. As between the species, some types of lesion vary, although the location is the same. In the lung, the rabbit shows perivascular inflammatory cell exudate with slight perivascular edema and an occluding proliferation of inflammatory cells within the lumen of both veins and arteries. The rat shows perivascular infiltration with inflammatory cells and some edema. The guinea pig shows a very marked perivascular edema and some perivascular inflammatory cell exudate and in addition, a marked thickening of the muscular coat of the arteries leading to final occlusion of the lumen. There is little or no proliferation within the lumen. The guinea pig additionally presents peribronchial inflammatory reaction. All 3 species show atelectasis of irregular areas in the lung. These various lesions are most intense and extensive in the guinea pig, least so in the rat, with the rabbit in the mid-position.

4. Up to a certain point the extent and intensity of the lesions increase with the amount of histamine administered. Beyond this, additional amounts of histamine do not increase the severity of the reaction. It is apparent that the animals develop a tolerance to histamine.

REFERENCES

1. Eppinger, H., Kaunitz, H., and Popper, H.: Die serose Entzündung, Vienna, 1935, Julius Springer.
2. Dammin G. J., and Bukantz, S. C.: Modification of Biologic Response in Experimental Hypersensitivity, J. A. M. A. **139**: 358-362, 1949.
3. Miller, C. P.: Spontaneous Interstitial Myocarditis in Rabbits, J. Exper. Med. **40**: 543-551, 1924.
4. Farmer, L.: Histamine in Anaphylaxis and Allergy, Bull. New York Acad. Med. **16**: 618-630, 1940. (Contains further references.)
5. Katzenstein, R.: Histamine Tolerance, Yale J. Biol. **16**: 325-331, 1944.

EFFECT OF FLAVONOID (VITAMIN P-LIKE) SUBSTANCES ON HISTAMINE TOXICITY, ANAPHYLACTIC SHOCK, HISTAMINE-ENHANCED CAPILLARY PERMEABILITY TO DYE, AND BLEEDING TIME; WITH DATA ON THE TOXICITY OF FLAVONOIDS*†‡

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WITH THE TECHNICAL ASSISTANCE OF MARY L. JORDAN, A.B.

A CONSIDERABLE literature has accumulated on the effects of flavonoids and related substances (vitamin P) on experimental and clinical sensitization phenomena. Most of the conflicting experimental work concerns histamine toxicity and anaphylactic shock.

INTRODUCTION

In 1941 in Japan, Hiramatsu[1] reported a favorable effect of "Hesperin,"§ a water-soluble flavanone glycoside, hesperidin-3′-ethylcarbonate[2, 3] on anaphylactic shock in the guinea pig. It was concluded that the drug in parenteral doses of 35 to 200 mg./kg. protected against anaphylactic shock if given before the sensitizing antigen, if given 30 minutes before or mixed with the shocking antigen, but not if given during the sensitization period.

In a second paper on the subject, Hiramatsu[4] examined the effects of the soluble hesperidin compound on anaphylactic shock in guinea pigs with their reticuloendothelial systems (RE) blocked with India ink. It was concluded that when single, large doses of the drug were injected 30 minutes before either the sensitizing or shock injections, additional protection was obtained over that caused by RE-blocking alone. When given repeatedly before or during the sensitization period, or when mixed with the shocking antigen, there was no effect.

From these two studies, Hiramatsu postulated that the drug in some way prevents "anaphylatoxins" or "histamine-like" substances, released in the presence of the shocking antigen, from dilating and increasing the permeability of the capillary blood vessels.

Hiramatsu's results were confirmed shortly by Fujihara[5] who, in addition, claimed that the protective effect was an indirect one since it was not elicited on the response of the isolated intestinal strip to histamine.

In 1942, Torii[6] examined the effect of the same soluble hesperidin compound on histamine shock in guinea pigs. It was claimed that protection ensued if 50 to 100 mg./kg. were given intraperitoneally daily for 5 days before,

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§Trade name, Takeda Company, Japan.

or intravenously 30 minutes before shocking with 0.21 mg./kg. histamine intracardially, which caused 87 per cent mortality in control animals. The number of animals used per group (8 to 10) and the histamine dose (see results of present paper) make these data convincing. It also was claimed that protection resulted if 50 mg./kg. were injected mixed with the histamine. The protective effect, though definite, was admitted to be of a low order, since if larger doses of histamine had been used, no protection would have been observed. Intravenous administration was claimed to be more effective than intraperitoneal.

In 1943, the German workers, Hüllstrung and Hack,[7] included in their more extensive studies[7, 8] of the antiallergic effect of "Citrin" on experimental skin sensitization by 2,4-dichlorobenzene, a study of Citrin on anaphylactic shock in the guinea pig. Citrin, first prepared and termed "vitamin P" by Szent-Györgyi,[9] is a water-soluble, crude flavonoid extract of vegetable origin, usually citrus, rose hips, or black currants. The flavonoid nature of such preparations has been thought by some to consist largely of "eriodictin," stated by Mager[10] to be eriodictyol-7-rhamnoglucoside. Hüllstrung and Hack presented data which suggested a protective effect of Citrin against anaphylactic shock. The results suggest a possible protection of a small order, by daily treatments with 15 mg. Citrin per animal, during the sensitization period. This conflicts with the results of Hiramatsu[1] with hesperidin-3′-ethylcarbonate. The data suffer the objection that insufficient numbers of animals were used (3 to 6 per group), which was admitted by the authors.

Two years later in France, Parrot and Richet,[11] working in support of their hypothesis that there is an antagonism between endogenous epinephrine (or sympathin) and histamine, and that vitamin P exerts its effects by potentiating the anticapillary fragility action of endogenous epinephrine (or sympathin), claimed that scorbutigenic diets enhance histamine toxicity in guinea pigs. Small doses (approximately 8 mg./kg.) of *epimerized d*-catechin (mixture of epimers presumably containing *d*-epicatechin, which Parrot believed to be the active epimer), returned this to normal. The drug was given 4 to 48 hours prior to shocking with histamine. The scorbutic animals, in addition to having an enhanced histamine sensitivity, also had an increased capillary fragility, which also was restored to normal or supranormal levels by the catechin injections.

In the same year, Ungar[12] developed an interesting and complex theory relating bleeding time and capillary fragility to histamine release by blood cells in vivo and in vitro by blood proteases. Using bleeding time as a correlate of this phenomenon, Ungar claimed to have found a remarkably high activity of "hesperidin methylchalcone"* and of *epimerized d*-catechin. His paper infers that this effect is mediated indirectly by a suppression of histamine release by the flavonoid substances studied.

In 1947, Wilson, Mortarotti, and De Eds[14] unsuccessfully attempted to demonstrate an antianaphylactic action of parenterally administered rutin in guinea pigs. They were, however, able to obtain results which demonstrated a

*"Hesperidin methylchalcone," California Fruit Growers Exchange, has not been crystallized nor its structure proved, but is believed to be a chalcone of hesperidin, with 2 or more hydroxyl groups methylated.[13]

slight but definite protective effect of rutin on histamine shock. Where 14 of 24 untreated controls died after 0.25 mg./kg. of intravenously administered histamine, only 2 of 17 died after intraperitoneal injection of 10 mg./kg. of 1 per cent rutin in 5 per cent ethanol 9 to 35 minutes before the histamine. No data were given which permit a quantitative assessment of the degree of protection conferred, since the histamine dose was not altered. In confirmation of Fujihara,[5] it was observed that the effect is not a direct antihistamine one, since there was no effect of rutin on the histamine responses of isolated intestinal strips or isolated, perfused lungs.

Independently, and in the same year, Raiman, Later and Necheles[15] failed to substantiate the histamine studies of Wilson, Mortarotti and De Eds. They claimed to find a protective effect of rutin against anaphylactic shock in guinea pigs, however. The rutin was administered intraperitoneally in alkaline solution or in propylene glycol in doses of approximately 2 to 5 mg./kg., 30 to 45 minutes prior to the shocking antigen. Whereas 16 controls all died, only one of 14 treated animals succumbed, and the one which did succumb was pretreated 60 minutes prior. (Wilson et al. claimed that the protective effect disappears after 60 minutes.) The shocking dose selected killed all the controls. It was not stated whether the shocking dose selected in each experiment was determined by titration on the same day.

Roth and Sheppard[16] repeated the work of Raiman et al. and in addition, used the soluble methylglucamine complex of rutin.[17] Under conditions in which 50 to 100 per cent of the control sensitized animals died on shocking, there was no effect of rutin administered in doses of 1-10 mg./kg. per animal, 10 to 45 minutes prior to shocking. In addition, Roth and Sheppard found no effect of the solubilized rutin on the mortality caused by an $L.D._{100}$ of histamine, which is not contradictory to the impressions gained by Torii[6] or to the data of Wilson et al.[14] The failure of this work to confirm Raiman et al. in regard to protection against anaphylactic shock by rutin again reopened the problem, particularly in view of the previous work by the Japanese and German investigators.

At about the same time, Levitan[18] also attempted to extend the purported "antihistamine" effect of rutin pretreatment to rabbits, using oral, intraperitoneal, intramuscular, and intravenous routes, both in single and in multiple doses up to and exceeding 700 mg./kg. Ten rabbits, pretreated with rutin by these various procedures, all succumbed to an "M.L.D." of intravenous histamine, as readily as the untreated controls. The objection is raised that in view of the results of Torii[6] and of Wilson et al.,[14] no protection was to be expected with doses of histamine much in excess of an $L.D._{50}$.

More recently, Arbesman and Neter[19] reported that rutin, even in massive doses given 15 to 60 minutes before (route not reported), had no effect on inverse anaphylaxis, Forssman anaphylaxis, or active anaphylaxis in rabbits and guinea pigs, using $L.D._{100}$ shocking doses of the various sera.

Included in this brief review should be mentioned the experiments of Lecoq, Chauchard, and Mazoué[20] who found that the chronaximetric effects of histamine and anaphylactic shock in the rat were suppressed by rutin and

epimerized d-catechin which also were claimed to antagonize acetylcholine. The interpretation of these findings is difficult in the absence of a more complete study.

The widely disparate results obtained by various investigators on the effects of flavonoids and related substances on histamine and anaphylactoid reactions seemed to demand a more critical assessment of these effects, particularly in view of several papers which have appeared recently[21-24] relating molecular structure to various biological actions of these compounds. An examination of these studies reveals that no common chemical structure is responsible for the activity of the compounds, and that a total lack of specificity exists. Accordingly, it is the purpose of the present paper to report experimental work bearing on these and related phenomena ascribed to flavonoids and related substances.

METHODS

1. *Histamine Shock.** The $L.D._{50}$ for histamine diphosphate, calculated as the free base, was determined in a series of 98 untreated guinea pigs. All animals were healthy, vigorous animals, body weight 300 to 900 grams, of

*A few remarks are in order on some of the preparations used in the histamine shock studies. Rutin is very insoluble in water but quite soluble in propylene glycol or at pH values above 10. If solubilized in these ways and then diluted[16, 27] or partially neutralized,[15] respectively, it will remain in solution while being injected. It also forms very soluble complexes with hexamethylenetetramine (HMT), a fact which was first observed in this laboratory, and with methylglucamine (MG).[16, 17] In our studies we used the proportions rutin: 4.7, HMT 0.94, water to 100; and rutin 4.6, MG 0.32, water to 100. Moderate heating causes the complex formation. The doses are expressed on the basis of free rutin. In all probability the complexes dissociate readily in the body, a subject which will be dealt with in another publication. The rutin used for these studies was obtained from Dr. W. G. Bywater, S. B. Penick Co., New York, N. Y.

Quercetin, the aglucone of rutin, is even more insoluble than rutin, but is solubilized by complexing with MG. We used the proportions: quercetin 2.5, MG 6.25, water to 100, with moderate heating. It also may be converted into a stable sulfonic acid derivative, quercetin-6′-sulfonic acid, which is about 1 per cent soluble in water at room temperature. The sodium salt was used in the present studies, and was prepared by the method of Watson and Sen[28] from the free acid which was supplied by Penick. The sulfonic group being in the 6′ position does not interfere with the free hydroxyls. The doses are expressed on the basis of free quercetin.

Quercitrin, the 3-rhamnoside of quercetin, is slightly more soluble than rutin, but must be solubilized by propylene glycol or by complexing with MG or HMT.

Gossypin, the 8-glucoside of gossypetin (3, 5, 7, 8, 3′, 4′-hexahydroxyflavone), is solub'e enough to administer without special treatment, although care must be exerted to avoid its ready autoxidation. It was obtained through the generosity of Professor T. R. Seshadri, Department of Chemistry, University of Delhi, Delhi, India.

Catechin and its epimers are flavanes, differing from flavones and flavanones in the absence of the carbonyl group. They are soluble enough in warm water but oxidize rapidly unless protected by nitrogen or a suitable antioxidant. Our *d*-catechin was prepared by the method of Clauser[29] from gum gambir by ether extraction and several recrystallizations. The *epimerized d*-catechin was prepared by the method of Freudenberg[30] by Hoffmann-La Roche, Inc., Nutley, N. J. and partially purified in our own laboratories. Its specific rotation was $[\alpha]_D^{26}$ = +17.8° to +23.2°, c = 1 per cent in 95 per cent ethanol, in the Hoffmann-La Roche samples (determinations made by Dr. J. Lee) and up to +38.5° in our own preparations (theoretical for *d*-epicatechin = +68.9° [31]).

The Hesperin is hesperidin-3′-ethylcarbonate, already described in the Introduction. It was obtained through the courtesy of Mr. Chobei Takeda XII, Takeda Pharmaceutical Industries, Ltd., Osaka, Japan, with the help of Gen. Crawford F. Sams, Public Health and Welfare Section, SCAP, Tokyo. We are indebted to both of these sources for this and other drug material and for the extensive translations of the Japanese literature which now is available from the Department of Commerce, as indicated in the bibliography.

The Citrin was prepared by the method of Szent-Györgyi[32] by Dr. Ernest Geiger for the California Fruit Growers Exchange. (Dr. Geiger prepared some of the Szent-Györgyi original extracts in Hungary.) It was lyophilized and stored as a powder.

The 2′,3,4-trihydroxychalcone was prepared by the S. B. Penick Co. under the direction of Prof. T. A. Geissman, Department of Chemistry, University of California at Los Angeles. It was solubilized by complexing with MG in the proportions: chalcone 2.5, MG 9, water to 100, with moderate heat. Its dose is expressed as the free chalcone.

The "Hughes and Parkes Cmpd. XII" is a soluble phosphate ester of a chalcone derivative, the synthesis of which is described by Hughes and Parkes[33] who also found it to have the highest anticapillary fragility activity of several soluble, synthetic compounds studied in their bioassays. It is the disodium salt of 3,4′-dihydroxy-4-methoxychalcone-4′-phosphate.

The esculetin-4-carboxylic acid was used as the soluble sodium salt of the monohydrate and was prepared in the laboratory of Dr. Geissman. It was used because it is a water-soluble derivative of esculetin, described by Lavollay[34] as having high anticapillary fragility activity and because of its high epinephrine potentiating activity in vitro, described by Clark and Geissman.[21]

mixed strains, and largely males. They were maintained on commercial rabbit diet, fresh oranges daily, and tap water for some time after obtaining them from the dealer. The histamine diphosphate was administered intracardially in the females and intravenously in the dorsal penile vein of the males. No anesthetic was used. The histamine diphosphate solutions were 55 to 110 mg. per cent in saline solution. The time of onset, severity, and duration of shock symptoms were not graded, since the impression was gained that only mortality data were reliable.

The $L.D._{50}$ values were calculated from dosage-mortality curves plotted from probits of the per cent mortality and logarithms of the doses, by Miller and Tainter's modification[25] of the method of Litchfield and Fertig.[26]

The various drugs were injected intraperitoneally, for the most part 30 minutes before the histamine injections. This time was selected because of the observations of Wilson et al.[14] and of Ambrose and De Eds[27] that the antihistamine and anticapillary permeability effects of parenterally administered rutin decrease rapidly after this time. The routes, solvents and schedules of administration are given in Table I of Results.

2. *Anaphylactic Shock.** The guinea pigs were sensitized by single intraperitoneal injections of sterile, undiluted horse serum, 0.25 to 0.50 c.c. per animal, or with 0.5 c.c. of fresh egg white, 12 to 26 days prior to shocking, these values varying between experiments. Anaphylactic shock was induced with appropriate dilutions of antigen as determined by titration in a few animals of the same sensitized group on the same day, before any drug studies were made. An untreated control group then was injected with this predetermined amount of antigen, followed by the drug injections in a second, treated group. The amount of shocking antigen selected by titration varied between 0.5 to 3.0 c.c./kg. of horse serum, depending on the size of the sensitizing dose and the duration of the sensitization period. When egg white was the antigen, the appropriate shocking dose was found to be 1 c.c./kg. of 1:100 filtered egg white in saline. The antigen was administered to the unanesthetized animals intra-

*In Experiment 1, rutin was given in 1 and 10 per cent solution in undiluted propylene glycol. In Experiment 2, it was suspended in saline, the suspension stabilized with sodium alginate. The preparation of the sodium salt of rutin acid succinate used in Experiment 3 is described by Haley, Clark, and Geissman.[35] It was used in 20 per cent aqueous solution.

The xanthorhamnetin (S. B. Penick Co.), the fairly soluble trirhamnoside of rhamnetin (7-methoxy derivative of quercetin) was made alkaline with NaOH, then readjusted to pH 8 with acetic acid, and used in 10 per cent solution in Experiments 3 and 4.

In Experiment 5, the rutin was solubilized by alkalinizing with NaOH and readjusting the pH to 8.5 with acetic acid just before use, by the method of Raiman et al.[15]

The hesperidin methylchalcone has been described in the Introduction. It was used as a 10 per cent aqueous solution in Experiment 6. It was supplied by the California Fruit Growers Exchange, Los Angeles.

The Citrin used in Experiment 7 differed from that used in the histamine experiments in being more refined. It was a highly soluble semipurified flavonoid derived from lemon peels, free of sugars and hesperidin, prepared by Dr. A. N. Wick in these laboratories. It was made from the residue remaining after evaporating a 50 per cent isopropyl alcohol extract of fresh lemon peel, supplied by the California Fruit Growers Exchange. A concentrated aqueous solution of the residue was refrigerated and the resulting precipitate, which was largely hesperidin, removed in an ultra-high speed refrigerated centrifuge. The supernatant was continuously extracted with ether to remove carotenoids and chlorophyll. To remove residual sugars and other impurities, the ether-extracted aqueous solution then was saturated with ammonium sulfate to render the water-soluble flavonoid less soluble, and extracted several times with *n*-butanol. Petroleum ether was added to the butanol layer which then was shaken several times with water. The separated water layer, containing the flavonoid was lyophilized to a golden powder which was almost indefinitely soluble in water. It was used in 10 per cent aqueous solution in the animal experiments.

The quercetin-6′-sulfonate used in Experiments 8 and 9 was used in 1.0 to 2.5 per cent warm aqueous solution.

The Hesperin used in Experiment 8 was used in 2.5 per cent aqueous solution.

The esculetin-4-carboxylic acid was used as a 0.5 per cent solution in saline, as the sodium salt.

venously in the dorsal penile vein and intracardially in a few cases (females). No differences were noted when the two routes were compared. In all cases, drug treatment was by intraperitoneal injection 30 minutes before shocking.

3. *Effect of Flavonoids on Extravasation of Intravenously Injected Evan's Blue (T-1824) into Histamine Skin Wheals.* Ambrose and De Eds[27] presented data indicating a delaying effect of rutin on the time of appearance of intravenously administered Trypan blue into histamine skin wheals. It was desired to attempt to extend this to include a comparison of rutin with a known, potent antihistaminic, and with a more soluble flavonoid which has a greater effect against histamine shock than rutin.

Large, male albino rats of the Slonaker strain, 180 to 250 grams body weight, were clipped and shaved on the dorsal side and injected intravenously in the tail vein with 0.01 c.c./kg. of 0.5 per cent Evan's blue (T-1824). Histamine diphosphate injections, 0.05 to 0.10 c.c. each, of serial, logarithmic dilutions in saline were given intracutaneously in duplicate sites down one side of the lightly ether-anaesthetized animal. The concentrations of histamine diphosphate used were 0 (saline controls), 0.0001, 0.001, 0.01, 0.1, and 1.0 per cent. The dye appeared in the wheals resulting from the higher concentrations of histamine in 5 to 15 minutes. The threshold of effect was considered to be that amount of histamine which evoked a blue wheal perceptibly more intense than the saline control wheals. This procedure was repeated in each rat at various time intervals after injecting the dye: immediately after, 30 minutes after, and 60 minutes after. In other rats this time was extended to 120 minutes. In all rats the threshold of effectiveness was found to be 0.001 per cent histamine diphosphate, equivalent to 1 microgram. Having established this threshold and the fact that the time interval between dye and histamine injections is not critical, experiments were designed to examine the possible effects of various drugs in altering this threshold.

As a typical antihistaminic, Pyribenzamine was selected. Two rats were injected with dye and the histamine threshold confirmed. Following this a large dose of Pyribenzamine, 75 mg./kg. was injected subcutaneously, and 20 minutes later the histamine threshold was redetermined.

In a third pair of rats, following the threshold determinations, which were 1 microgram as before, 25 mg./kg. of 2.5 per cent warm aqueous solution of sodium quercetin-6′-sulfonate were injected intravenously in the tail vein. Fifteen minutes later, the histamine thresholds were redetermined.

In a fourth pair of animals, a similar experiment was performed, using rutin as the drug. Rutin, 100 mg./kg. of a 10 per cent solution in propylene glycol, was injected intraperitoneally and the histamine thresholds redetermined 30 minutes later.

4. *Effect of Flavonoids on Bleeding Time.* Ungar[12] and Parrot and Galmiche[36] have described a decrease in bleeding time in guinea pigs after injection of "hesperidin methylchalcone" and *epimerized d*-catechin. Ungar[12, 37] used bleeding time as a correlate with histamine release by blood proteases, as mentioned in the Introduction. He inferred that the flavonoids used interfered with this release. It seemed important, therefore, to attempt to confirm and extend his observations.

Ungar's method[37] was followed carefully for determining bleeding time in guinea pigs. The effects of several flavonoids on bleeding time were studied, giving the drugs both intraperitoneally and intravenously in doses from 0.0001 mg./kg. to 100 mg./kg., both in unanesthetized and in nembutalized animals. The bleeding time determinations were made before and from 30 minutes to 4 hours following the drugs. In several larger groups of animals, one group served as untreated controls and the others as treated, and bleeding time determinations were made in all cases after but not before the drug. The drugs included: hesperidin methylchalcone, Citrin (same type used in anaphylactic shock experiments), rutin, sodium salt of quercetin-6′-sulfonic acid, *d*-catechin, *l*-epicatechin* and *epimerized d*-catechin ($[\alpha]_D^{25} = +38.5°$, 1 per cent in 95 per cent ethanol).

5. *Toxicity Studies.* During the course of the present and other investigations, it became necessary to obtain some impressions of the acute and chronic oral and parenteral toxicity of various flavonoids in several species of animals. The data will be presented in Results.

RESULTS

1. *Histamine Shock.* The $L.D._{50}$ of intravenously administered histamine diphosphate, calculated as the free base, was calculated from the data on 98 animals shown in Table I, to be 0.125 ± 0.015 mg./kg. This figure is somewhat lower than the figure, 0.296 found by Giscafre and de Espanes[38] for intracardially injected histamine in 210 guinea pigs. Dietary, environmental, and animal strain differences may account for the slight differences in these and other published figures.

Similar data were obtained on 58 animals pretreated with 7.5 mg./kg. quercetin-6′-sulfonic acid (10 mg./kg. of the sodium salt) 30 minutes before shocking. These data also appear in Table I.

TABLE I. TOXICITY OF HISTAMINE IN UNTREATED AND QUERCETIN SULFONATE-TREATED GUINEA PIGS

	UNTREATED			TREATED*		
HISTAMINE DOSE (MG./KG.)	NO. GUINEA PIGS	NO. DIED	% DIED	NO. GUINEA PIGS	NO. DIED	% DIED
0.10	10	0	0			
0.15	10	1	10			
0.20	30	19	63	10	0	0
0.25				19	2	11
0.30	28	24	86	10	2	20
0.40	9	8	89	9	3	33
0.50	11	11	100	10	8	80
Total	98			58		

*7.5 mg./kg. I.P. 30 mins. before histamine.

From the data in Table I the $L.D._{50}$ for the drug-treated animals was calculated to be 0.425 ± 0.032 mg./kg.

The data for the untreated controls and the quercetin sulfonate treated animals which appear in Table II were taken from the mortality probit-logarithmic dose curves.

*Generously prepared and supplied by Professor T. R. Seshadri.

TABLE II. EFFECT OF FLAVONOIDS ON HISTAMINE SHOCK IN GUINEA PIGS

HISTAMINE DOSE, MG./KG.			0.20		0.25		0.30		0.40		0.50		0.60		
TREATMENT			MORTALITY												L.D.$_{50}$
DRUG	MG./KG.	SOLUTION	FR.[a]	%	FR.	%	FR.	%	FR.	%	FR.	%	FR.	%	MG. KG.
Controls (98)		Untreated		44		64		89		93		100			0.21 n = 98
Rutin	10	0.5% alk. soln.			6/10	60									
	50	1.7% in P.G.[b]	2/4	50					5/5	100					
	100	5.0% in P.G.	1/10	10					4/10	40			10/10	100	0.31
	100	3.3% in P.G.[c]	2/5	40			2/3	67	3/6	50					n = 89
	85	HMT[d]			3/10	30			7/10	70					
	100	HMT							8/10	80					
	100	MG[e]							7/10	70					
Quercetin	10	MG	3/10	30					6/10	60					
	50	MG							7/8	88					
Quercetin-SO_3Na (58)[f]	7.5			0		10		20		44		66			0.43 n = 58
Quercitrin	10	1.25% in P.G.	4/10	40											
	50	1.25% in P.G.	3/8	37											
Gossypin	100	2.5% aq.							8/10	80					
d-catechin	10	1.25-5% in P.G.	2/8	25					4/4	100					
	50	1.25-5% in P.G.	3/8	38											
Epimerized d-catechin	10	0.5% in saline			5/10	50									
Hesperin	10	0.5% in saline			4/10	40									
	100	2.5% aq.			6/10	60									
Citrin	50	4% aq.	7/14	50			3/4	75	4/4	100					
2′,3,4-chalcone[g]	10	MG							9/10	90					
H & P Cmpd. # XII[h]	100	2.5% aq.			6/10	60									
Esculetin-4-COONa	10	2.5% aq.			7/9	78									

Key: Drugs administered intraperitoneally 30 minutes before histamine, unless otherwise noted.
PG = 50 per cent warm propylene glycol.
a = Fraction dead (number dead/total used).
b = Intravenous instead of intraperitoneal.
c = 5 hours before instead of 30 minutes.
d = Hexamethylenetetramine complex. See Methods.
e = Methylglucamine complex. See Methods.
f = 0.5–1.25 per cent aqueous solution.
g = 2′,3,4-trihydroxychalcone.
h = Hughes & Parkes' Cmpd. XII, disodium salt of 3,4′-dihydroxy-4-methoxychalcone-4′-PO_3.

Table II summarizes the results obtained for the flavonoid drug treatments in 416 guinea pigs. Descriptions of the drugs have been given in Methods.

It is evident from the results in Table II that none of the flavonoids had a significant effect on the toxicity of histamine except rutin and quercetin-6′-sodium sulfonate. There is a slight order of protection conferred by the various forms of rutin administered in doses of 100 mg./kg. but not of 50. The degree of protection of 100 mg./kg. rutin is such that the $L.D._{.50}$ of histamine is of the order of 1.5 times that in the untreated animals. From Tables I and II it is evident that quercetin-6′-sulfonate was the only flavonoid used which is effective in reasonably small doses, 7.5 mg./kg. (expressed as free acid). The degree of protection conferred is such that the $L.D._{.50}$ of histamine is 2 times that in the untreated animals. Since the dosage used was 10 mg./kg., expressed as the sodium salt, this compound is much more effective than the various forms of rutin used, but still is too low to be of importance as a true antihistaminic, some of which are said to protect against several hundred fatal doses of histamine.

2. *Anaphylactic Shock.* The drugs and solutions used are described in Methods. The results on 177 guinea pigs are summarized in Table III. Over 250 animals were used, including the preliminary titrations essential for each experiment.

TABLE III. EFFECT OF FLAVONOIDS ON ANAPHYLACTIC SHOCK IN GUINEA PIGS*

EXPERIMENT NUMBER	TREATMENT		MORTALITY	
	DRUG[a]	DOSAGE MG./KG.	FRACTION DEAD	% DEAD
1.	Untreated		7/9	78
	Rutin in P.G.	6	3/4	75
	Rutin in P.G.	100	2/4	50
2.	Untreated		4/5	80
	Rutin (suspension)	200	3/6	50
3.	Untreated		8/10	80
	Na-RAS[b]	200	3/4	75
	Xanthorhamnetin	200	5/11	46
4.	Untreated		6/8	75
	Xanthorhamnetin	50	6/8	75
5.	Untreated		7/11	63
	Rutin (alkaline)	100	4/11	33
6.	Untreated		4/6	70
	HMC[c]	100	4/8	50
7.	Untreated		4/5	80
	Citrin	100	6/6	100
8.	Untreated		10/10	100
	Quercetin-SO_3Na[d]	25	7/8	87
	Hesperin	100	5/5	100
9.	Untreated		8/17	47
	Quercetin-SO_3Na	10	5/17	30
10.	Untreated		7/12	58
	Esculetin-4-COONa	10	10/12	83

*Drugs administered intraperitoneally 30 minutes before shocking unless otherwise noted.

Key: a = See Methods.
b = Sodium salt of rutin acid succinate.
c = "Methylated hesperidin chalcone."
d = Intravenously.

An examination of the results of the rutin Experiments 1, 2, 3, and 5, reveals that there may be a protective effect of doses in excess of 100 mg./kg. since in all 4 experiments the mortality was slightly less than in the controls. This is most apparent in Experiment 5, in which the number of animals used was sufficient to demonstrate a trend. No particular significance is attached to the experiments in which the numbers of animals were smaller than this, but they are included for comparative purposes. The effect, if indeed existent at all, is of a very slight order and is absent in the case of the other flavonoids studied, including the quercetin-6′-sulfonic acid, which had a protective effect in histamine shock. Further experiments involving larger numbers of animals were not deemed justified from these data in general which were not considered significant.

3. *Extravasation of Intravenously Injected Evan's Blue (T-1824) Into Histamine Skin Wheals.* Pyribenzamine, 75 mg./kg. administered subcutaneously, increased the threshold of effectiveness of intracutaneous histamine diphosphate from 1 to greater than 1000 micrograms.

Quercetin-6′-sulfonate, 25 mg./kg. intravenously administered, and rutin, 100 mg./kg. intraperitoneally administered, had no effect on the histamine thresholds.

4. *Bleeding Time.* Ungar's frequency-distribution curve for bleeding time in guinea pigs[37] was faithfully reproduced, with an arithmetic mean bleeding time of 130 seconds in comparison with Ungar's figure of 131, and that of 130 seconds obtained by Parrot and Galmiche.[36] Upon administration of the same drugs, "hesperidin methylchalcone" and *epimerized d*-catechin, and using the same dosage-time schedules that Ungar, and Parrot and Galmiche used, at first it was believed that a significant decrease in bleeding time was being observed, for example, from 130 to 100 seconds. Subsequently, however, it was found that when the investigators and two trained, skilled technicians were given coded, unknown solutions including saline blanks, there was no effect on either the shape of the distribution curve or the arithmetic mean bleeding times, using 4 determinations per animal and 4 to 10 animals per group. Several thousand determinations of bleeding time were made in the course of this work.

5. *Toxicity Studies.*

(A) *Oral Administration.*—None of the flavonoids studied were toxic when orally administered in single, large doses up to 0.2 to 0.5 Gm./kg., except quercetin-6′-sulfonate which induced diarrhea in fasted but not unfasted rats. The compounds studied in rats, mice, and guinea pigs were: rutin, rutin methylglucamine complex, rutin hexamethylenetetramine complex, sodium rutin acid succinate, sodium rutin acid phthalate,[35] "calcium flavonate glucoside,"[39] Citrin, quercetin, gossypin, xanthorhamnetin, and the sodium salt of esculetin-4-carboxylic acid.

Single oral doses up to 100 mg./kg. (10 Gm. in one individual) of rutin, hesperetin, "calcium flavonate", Citrin and "methylated hesperidin chalcone" gave no symptoms in human subjects.

Chronic toxicity in the rat was observed only in the case of 2′,3,4-trihydroxychalcone at a level of 1 per cent in the diet, which caused a 10 to 15 per cent loss in body weight within a week in 10 gram rats. All the other soluble flavonoids studied were nontoxic at levels of 0.1 to 0.5 per cent in the drinking water, and in the case of the insoluble compounds, up to 1 per cent in the diet.

(B) *Parenteral Administration.*—None of the flavonoids listed in the histamine and anaphylaxis studies were toxic in the single, intraperitoneal doses given except the quercetin-6′-sodium sulfonate which will be discussed presently. When administered intravenously the picture was different. Quercetin methylglucamine (5 per cent quercetin, 12.5 per cent methylglucamine in water) caused prostration and convulsions followed by death when injected intravenously in guinea pigs in doses exceeding 50-100 mg./kg. The methylglucamine complex of 2′,3,4-trihydroxychalcone behaved similarly.

On repeated intraperitoneal doses of sterile, aqueous, alkalinized rutin, pH 10.3, in doses exceeding 100 to 150 mg./kg., weight loss was observed in guinea pigs which did not occur when an equivalent amount of NaOH was given. In doses of 25 mg./kg. twice daily, however, no such effect was observed.

Xanthorhamnetin in 10 per cent sterile aqueous solution, pH 10.0, was well tolerated subcutaneously in guinea pigs at doses of 50 to 150 mg./kg. twice daily for 2 weeks.

Gossypin and the sodium salt of esculetin-4-carboxylic acid monohydrate were nontoxic at doses of 50 mg./kg. intraperitoneally for 10 days in rats.

The sodium salt of quercetin-6′-sulfonic acid was the most toxic substance studied. Doses exceeding 10 mg./kg. intraperitoneally in guinea pigs, and 25 mg./kg. in rats, caused distress and larger doses caused prostration with recovery in 30 to 60 minutes. Intravenously, in mice, guinea pigs, rats, and rabbits, prostration followed by death occurred at doses of 100 mg./kg. or more, depending upon the rate of injection. By the method of Miller and Tainter,[25] the $L.D._{50}$ on intravenous injection in 15 guinea pigs was 26.3 ± 0.6 mg./kg. of a 1 per cent solution in saline, pH 7.0; and intraperitoneally in 37 rats, average body weight 150 grams, it was 125 ± 1.7 mg./kg. Autopsies after death by intraperitoneal injection revealed marked congestion of the peritoneum and visceral organs, especially of the gastrointestinal tract. Following intravenous injection, death occurred by respiratory failure, with occasional pulmonary hemorrhage and the heart stopped in diastole.

As the free acid, quercetin-6′-sulfonic acid is soluble in water at 25° C. to the extent of 4.2 Gm. per 100 c.c. As the sodium salt at pH 7.0, its solubility is 0.94, but at 40-45° C. it is soluble up to 2.5 per cent.

DISCUSSION

The results do not confirm those of Hiramatsu,[1] Fujihara,[5] or Torii[6] in histamine and anaphylactic shock, with hesperidin-3′-ethylcarbonate (Hesperin) obtained from the same source, although Torii's method of intravenous injection was not used. Citrin had no effect on anaphylactic shock when given 30 minutes before shocking although it is admitted that the time of injection differed from

Hüllstrung and Hack's[7] method of daily administration during the sensitization period. In confirmation of Wilson et al.,[14] rutin was found to have a very slight protective effect in histamine shock, which also confirms Roth and Sheppard,[16] and Arbesman and Neter,[19] but not Raiman et al.[15]

Quercetin-6′-sodium sulfonate gave more protection against histamine shock than 10 times as much rutin and it would have been interesting perhaps to have seen what repeated doses before and during the senstitization period of anaphylaxis would have done, inasmuch as it had no effect when given once 30 minutes before shocking, in contrast to its protective effect in histamine shock. The protection conferred, however, is far less than that obtained with the "specific" antihistaminics such as Pyribenzamine, Neoantergan, Benadryl and related compounds, a point which already has been made by Wilson et al.[14] Their interpretation of the histamine protective effect was that there may be an endogenous epinephrine protective or synergistic effect of the flavonoids. This is not supported by the present findings in view of our previous studies,[21] in which the potentiation of the action of epinephrine on isolated, smooth muscle showed the activity series: quercetin-6′-sulfonic acid>quercetin>2′,3,4-trihydroxychalcone>gossypin>esculetin>rutin>the catechins (?)> Citrin; and that the other flavonoids which are mentioned in the present work were devoid of activity. In the present study, the absence of activity of gossypin, the catechins, 2′,3,4-trihydroxychalcone, and the soluble esculetin derivative do not support a correlation of epinephrine potentiation with the histamine protective effect.

The absence of an effect of the quercetin-6′-sulfonate in anaphylactic shock, together with the fact that it was the most toxic substance examined, suggests a lack of specificity and a "stress" response in the antihistamine action. This recalls the extensive review of Hill and Martin[40] who have compiled reports of a large number of chemically unrelated drugs which ameliorate or prevent anaphylactic shock. It also recalls the study of Randall and Sevringhaus[24] who found a total lack of chemical specificity in the ability of several drugs to exert an anticapillary fragility action in experimental purpura.

In view of the literature quoted and the present results on the slight effects of one or two flavonoids on histamine but not anaphylactic shock, one wonders if the antihistamine effect might not be due to a stress-induced epinephrine release from the adrenal similar to that induced by the administration of other toxic agents such as nicotine and even possibly an adrenocorticotrophic-corticosteroid release* rather than any specific effect on epinephrine potentiation, "capillary permeability," hyaluronidase, histamine release mechanisms, and so on.

The present studies also do not support the data of Wilson et al.[14] that rutin inhibits the extravasation of intravenous dye into histamine wheals nor the results of Ungar[12] and of Parrot and Galmiche[36] on the effects of flavonoids on bleeding time. This is in agreement with Randall[41] who obtained the same results on bleeding time that are reported in the present study.

*It recently has been shown that 10 mg./kg. of intraperitoneally administered quercetin-6′-sodium sulfonate causes as much decrease of adrenal ascorbic acid as 0.2 mg./kg. of epinephrine or arterenol in intact rats.

In conclusion, it is the opinion of the writers that the flavonoids have no specific effects on anaphylactic or histamine shock, even though one of them, quercetin-6′-sulfonate, may double the amount of histamine required to give the $L.D._{50}$.

SUMMARY

1. The $L.D._{50}$ of intravenously administered histamine diphosphate (expressed as free base) in 98 guinea pigs, was 0.21 mg./kg. The $L.D._{50}$ of histamine given 30 minutes after the intraperitoneal administration of 100 mg./kg. of various types of solubilized rutin was 0.31 mg./kg. in 89 guinea pigs, or 1.5 times greater than in the untreated animals. Smaller doses of rutin had no effect. Thirty minutes after 7.5 mg./kg. of quercetin-6′-sodium sulfonate (expressed as free quercetin), the $L.D._{50}$ was 0.43 mg./kg. in 58 guinea pigs, or 2.0 times that of the controls and greater than that caused by 10 times as much rutin.

Other flavonoid substances tested were inactive in doses of from 10 to 100 mg./kg. intraperitoneally administered 30 minutes before various doses of histamine down to 0.20 mg./kg. The inactive drugs were: quercetin methylglucamine, quercitrin, gossypin, *d*-catechin, *epimerized d*-catechin, hesperidin-3′-ethylcarbonate (Hesperin), Citrin, 2′,3,4-trihydroxychalcone-methylglucamine, the sodium salt of esculetin-4-carboxylic acid and the disodium salt of 3,4′-dihydroxy-4-methoxychalcone-4′-phosphate.

2. Doses of rutin of 100 mg./kg. or greater, intraperitoneally administered 30 minutes prior to shocking, had a doubtful or negligible effect on anaphylactic shock in guinea pigs. Doses of from 10 to 200 mg./kg. of the other flavonoids studied had no effect. These included: the sodium salt of rutin acid succinate, xanthorhamnetin, "methylated hesperidin chalcone," Citrin, the sodium salt of quercetin-6′-sulfonic acid, and the sodium salt of esculetin-4-carboxylic acid.

3. Pyribenzamine increased by a thousand-fold the amount of intracutaneous histamine necessary to cause extravasation of intravenously administered Evan's blue in rats, whereas 100 mg./kg. of intraperitoneally administered rutin, or 25 mg./kg. of intravenously administered quercetin-6′-sodium sulfonate, had no effect.

4. Flavonoid substances had no effect on bleeding time in guinea pigs under the experimental conditions employed.

5. Some data are given on the toxicity of oral and parenteral, acute and chronic administration of several flavonoid substances.

REFERENCES

1. Hiramatsu, N.: Studies on Vitamin P (Hesperidin). IV. Effect of Hesperidin on Serum Anaphylaxis, Jap. J. Dermatol. & Urol. **49**: 304-311, 1941 (in Japanese). German abstract on p. 41 of Abstract Section; English Abstract in Far East Sc. Bull. **1**: 37, 1941. For full English translation see: Biblio. Sc. Indust. Rep. **10**: 204, 1948; PB Report No. 92310, 18 pp., Library of Congress, Photoduplication Service, Pub. Board Project, U. S. Dept. Commerce, Washington 25, D. C.
2. Ohta, M.: Therapeutic Hesperdin Compounds, U. S. Patent No. 2,350,804, 1944.
3. Takeda Pharmaceutical Industries, Ltd., Osaka, Japan: Personal communications.

4. Hiramatsu, N.: Studies on Vitamin P (Hesperidin). V. Effects of Hesperidin on Serum Anaphylaxis When the Reticuloendothelial System Is Blocked, Jap. J. Dermatol. & Urol. **50:** 37-45, 1941 (in Japanese). For full English translation see Biblio. Sc. Indust. Rep. **10:** 204, 1948; PB Report No. 92311, 21 pp. (See reference 1.)
5. Fujihara, J.: J. Osaka Med. Assoc. 1942. Quoted by Torii. (See reference 6.)
6. Torii, S.: Supplement to the Study of Vitamin P (Hesperidin). I. The Effect of Hesperidin on Histamine Shock, J. Osaka Med. Assoc. **42:** Special edition No. 1, 1943 (in Japanese). For full English translation, see Biblio. Sc. Indust. Rep. **10:** 207, 1948; PB Report No. 92315, 15 pp. (See reference 1.)
7. Hüllstrung, H., and Hack, K.: Untersuchungen über die antiallergische Wirkung von Vitamin P (Citrin). II. Mitteilung, Ztschr. f. Immunitätsforsch. u. exper. Therap. **103:** 327-344, 1943.
8. Hüllstrung, H., and Hack, K.: Experimentelle Sensibilisierung und deren Beeinflussung durch Vitamine beim Meerschweinchen, Ztschr. f. Immunitätsforsch. u. exper. Therap. **100:** 393-428, 1941.
9. Armentano, von L., Bensath, A., Beres, T., Rusznyak, I., and Szent-Györgyi, A.: Ueber den Einfluss von Substanzen der Flavongruppe auf die Permeabilität der Kapillaren, Deutsche med. Wchnschr. **62:** 1325-1328, 1936.
10. Mager, A.: Ueber die Zusammensetzung des Eriodictyolglykosides, Ztschr. f. physiol. Chem. **274:** 109-115, 1942.
11. Parrot, J., and Richet, G.: Accroisement de la sensibilitè a l'histamine chez le cobaye sourmis a un régime scorbutigine; reduction de cet accroisement par l'administration de vitamine P, Compt. rend. Soc. de biol. **139:** 1050-1053, 1945.
12. Ungar, G.: Action of Drugs on Bleeding Time, J. Physiol. **103:** 180, 1944; Endocrine Function of the Spleen and its Participation in the Pituitary-Adrenal Response to Stress, Endocrinology **37:** 329-340, 1945.
13. Wilson, C. W.: Alkylated Chalcone Derivatives, U. S. Patent No. 2,425,291, 1947.
14. Wilson, R. H., Mortarotti, T. G., and De Eds, F.: Some Pharmacological Properties of Rutin, J. Pharmacol. & Exper. Therap. **90:** 120-127, 1947.
15. Raiman, R. J., Later, E. R., and Necheles, H.: Effect of Rutin on Anaphylactic and Histamine Shock, Science **106:** 368, 1947.
16. Roth, L. W., and Sheppard, I. M.: Absence of Protective Action of Rutin in Anaphylactic and Histamine Shock, Science **108:** 410-411, 1948.
17. Plungian, M. B.: Solubilization of Rutin with Methylglucamine, U. S. Patent No. 2,451, 772, 1949.
18. Levitan, B. A.: Rutin in Histamine Shock, Proc. Soc. Exper. Biol. & Med. **68:** 569-571, 1948.
19. Arbesman, E. E., and Neter, E.: The Effect of Pyribenzamine and/or Rutin on Inverse Anaphylaxis, Forssman Anaphylaxis and Active Anaphylaxis, J. ALLERGY **20:** 80, 1949.
20. Lecoq, R., Chauchard, P., and Mazoué, H.: Les vitamines antagonistes de l'action nerveuse de l'histamine et du choc anaphylactique, Compt. rend. Acad. d. Sc. **227:** 1264-1266, 1948.
21. Clark, W. G., and Geissman, T. A.: Potentiation of Effects of Epinephrine by Flavonoid ("Vitamin-P"-like) Compounds. Relation of Structure to Activity, J. Pharmacol. & Exper. Therap. **95:** 363-381, 1949.
22. Wilson, R. H., and De Eds, F.: The In Vitro Protection of Epinephrine by Flavonoids, J. Pharmacol. & Exper. Therap. **95:** 399-406, 1949.
23. Salva, J., Neumann, J., and Lavollay, J.: Action de quelques substances antifragilité vasculaire sur l'hypertension adrénalinique chez le chat spinal, Compt. rend. Acad. d. Sc. **227:** 1170-1172, 1948.
24. Randall, L. O., and Sevringhaus, E. L.: Effect of "Vitamin P"-like Substances on Capillary Resistance in Thrombocytopenic Purpura in Rats, Arch. Biochem. **22:** 132, 1949.
25. Miller, L. C., and Tainter, M. L.: Estimation of the ED_{50} and its Error by Means of Logarithmic-Probit Graph Paper, Proc. Soc. Exper. Biol. & Med. **57:** 261-264, 1944.
26. Litchfield, J. T., Jr., and Fertig, J. W.: On Graphic Solution of Dosage-Effect Curve, Bull. Johns Hopkins Hosp. **69:** 276-286, 1941.
27. Ambrose, A. M., and De Eds, F.: Effect of Rutin on Permeability of Cutaneous Capillaries, J. Pharmacol. & Exper. Therap. **90:** 359-363, 1947.
28. Watson, E. R., and Sen, K. B.: Dyes Derived from Quercetin, J. Chem. Soc. **105:** 389-399, 1914.
29. Clauser, R.: Beitrag zur Kenntniss des Catechins, Ber. d. deutsch. chem. Gesellsch. **36:** 101-107, 1903.
30. Freudenberg, K., Fikentscher, H., Harder, M., and Schmidt, O.: Die Umwandlung des Cyanidins in Catechin; 20th Mitt. über Gerbstoffe und ähnliche Verbindungen, J. Liebig's Annalen d. Chemie **444:** 135-145, 1925.

31. Freudenberg, K., and Purrman, L.: Raumisomere Catechins. IV. 16th Mitt. über Gerbstoffe und ähnliche Verbindungen, J. Liebig's Annalen d. Chemie **437:** 274-285, 1924.
32. Szent-Gygörgyi, A.: Methoden zur Herstellung von Citrin, Ztschr. f. physiol. Chem. **255:** 126-131, 1938.
33. Hughes, E. G., and Parkes, M. W.: Studies on Dietary Factors Affecting Capillary Resistance, E. C. Barrell Jubilee Volume, Hoffmann-La Roche, Inc., F. Reinhardt, Basle, 1946, pp. 216-228; U. S. Patent No. 2,465,320.
34. Lavollay, J.: Sur la vitamine P. Action de l'esculoside et de l'esculétol sur la résistance des capillaires, Compt. rend. Soc. de biol. **139:** 270-272, 1945.
35. Haley, T. J., Clark, W. G., and Geissman, T. A.: Studies on "Vitamin P." I. Topically Applied "Vitamin P"-like Substances on the Mammalian Capillary Bed, Proc. Soc. Exper. Biol. & Med. **65:** 202-207, 1947.
36. Parrot, J. L., and Galmiche, P.: Action de la vitamine P sur le temps de saignement, Compt. rend. Soc. de biol. **139:** 948-950, 1945.
37. Ungar, G.: Études biochimiques et physiologiques sur deux substances actives produites par la rate, J. de physiol. **39:** 219-248, 1947.
38. Giscafre, L., and de Espanes, E. M.: Toxicity of Histamine for the Guinea Pig, Arch. internat. de pharmacodyn. et de thérap. **77:** 300-302, 1948.
39. Clark, W. G., Uncapher, R. P., and Jordan, M. L.: Effect of Flavonoids (Vitamin P) on Mortality from Total Body Irradiation, Science **108:** 629-630, 1948.
40. Hill, J. H., and Martin, L.: A Review of Experimental Studies of Non-specific Inhibition of Anaphylactic Shock, Medicine **11:** 141-223, 1932.
41. Randall, L. O.: Hoffmann-La Roche, Inc., Nutley, New Jersey, personal communication.

TREATMENT OF RESPIRATORY ALLERGY IN CHILDREN BY ORAL ADMINISTRATION OF DUST AND POLLEN EXTRACTS

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THE history of oral therapy of hay fever goes back to 1922,[1] but no serious attention was given to it until 1927.[2] Since that time a considerable number of reports has accumulated, some of them claiming excellent results, some poor, and others taking a middle ground. Some have abandoned the method after brief trial while others continue to use it. It can be said that the method has not been adopted by many workers in this field. It would seem evident that the results have not been good enough for this method to supplant hypodermic therapy.

Work has been reported which has cast doubt upon the absorption of pollen antigen from the intestinal tract, at least in discoverable amounts.[3] Attempts have been made by some to protect the antigen from the action of the gastric juice, and greater efficiency in treatment has been claimed.[4] Our own work, done some years ago,[5] convinced us that absorption of antigen did take place and more recent reports have confirmed this.[6] In our work we have made no effort to protect the antigen from the action of the gastric juice other than that protection afforded by wide dilution and administration on a fasting stomach. Since all our work has been done with extracts while the work of others, with few exceptions, has been done with dry pollen, we have wondered whether the marked differences in absorption which have been claimed may be explained, at least partly, by this difference.

While our results with oral treatment, using pollen, were by no means as satisfactory as by hypodermic treatment, there were patients who secured satisfactory relief, and the questions raised by the failure of many others have served to maintain our interest in the subject. Also, over a period of several years, we have used the oral method of desensitization to the basic foods and have found the percentage of satisfactory results quite high.[7] Added to this, the reluctance of many parents and most children to undertake prolonged hypodermic treatment made us consider the oral method of treatment in children. The evidence that absorption of unchanged antigen may be much greater in children than in adults[6, 8] added impetus to the plan to study a considerable group of children suffering from allergy of the respiratory tract.

Because, in our experience, perennial, nonpollen allergy is much more frequent in children than is pollen allergy, it was decided, in this investigation, to use house dust as the antigen in dust-sensitive children and to include in the study asthma as well as perennial and seasonal hay fever. As our figures will show, the number receiving house dust antigen was many times greater than the number who had pollen therapy.

This study has been under way for four years. The patients were not selected. All children under 13 years of age and in whom dust or pollen was believed to be the primary etiologic factor in the production of a respiratory allergy were placed on oral treatment. Those whose pollen sensitivity was only a part of a perennial nonpollen hay fever or asthma are not included in this study. If this treatment proved satisfactory, it was continued. If the result was not good, hypodermic treatment was advised.

Pollen treatment was done with the 1:50 glycerosaline extract identical with that used in hypodermic therapy. Dust therapy was done with a concentrated glycerosaline extract and it too was the same as that used in hypodermic therapy.

The first dose was one drop from a medicine dropper delivering approximately one minim. This was dropped into 6 to 8 ounces of water, milk, orange juice, or other beverage, stirred and drunk between meals. The second dose was 2 minims given in the same way on the second day and one minim was added to the dose each day until 15 were taken on the fifteenth day. Regardless of the number of drops, they were added to the beverage at once so that the patient took only one dose a day. Even 15 drops when added to 6 to 8 ounces of beverage do not noticeably alter the taste of the drink and have not, in our experience, even in a single instance, produced any gastrointestinal symptoms. After reaching a dose of 15 drops this amount was continued as the daily dose. The patient was given a 15 c. c. bottle and this served as a satisfactory check on the size of the drop delivered since, when the fifteenth dose was taken, one-half the contents of the bottle should have been used. If, at this time, the bottle was not half empty, it indicated that the drops were too small and the dosage was continued until as much as 20 drops were taken.

It has been found that it is unnecessary to begin treatment with doses smaller than one minim of the 1:50 dilution of pollen or the concentrated dust extract. These initial doses, when properly diluted, have not at any time caused any symptoms of any kind. Relief of symptoms also seemed to proceed as well with these doses as with a much smaller initial dose and a much more gradual increase. We have found, too, almost without exception, that it is of no benefit to increase the number of drops beyond 15. This is an arbitrary number but we have found that children who do not show definite improvement on this dosage will rarely show it on any larger amount.

In the earlier months of this work we gave an antacid with the antigen but soon found it of no importance and have not used it since.

After a dose of 15 drops was reached, if the patient was free from symptoms, this dose was continued daily as a maintenance dose. After a few weeks, if the child remained well, the dose was given only every second or third day to see if relief could be maintained on this basis. Usually a daily dose was required.

After a dose of 15 drops was reached, if the patient was not relieved, this dose was continued daily for one week and then, if there was no marked improvement, this form of treatment was stopped and hypodermic treatment advised.

Oral treatment, using dust or pollen, was given to 402 children. Of these, 108 did not have follow up reports and were excluded from this study. This failure to have adequate records on approximately one-fourth of our patients is a sad commentary on the conditions under which we worked during and just following the war. We have been able to get recent reports from a few of these patients and they indicate that some of the results were good while others were not, but we still lack sufficient information on most of this group and so are unable to use them for this record.

There were 73 children who were believed to be sensitive to dust and also to another inhalant or to some food. These were given dust extract orally and appropriate treatment for their other sensitivity. Since it was impossible to know the relative importance of the dust and the other allergens in the production of symptoms, it was not possible to know just what evaluation to place upon the dust therapy. Of this group 67 were classified as getting good results while only 6 were poor. These are better results than were obtained with oral treatment alone which would lead us to believe that the other treatment these patients received contributed a great deal to the result. Since the value of oral treatment in these patients cannot be accurately determined, these figures are kept separate from the others of the group as they appear in the tables.

Table I presents the results of oral therapy in 221 children. They have been grouped according to the antigen with which they were treated and as to the results obtained. Since the results reported are the statements of the parents they must be recognized as approximations and, in all probability, not always objective observations. For this reason we have not attempted to express the results as "Excellent," "Good," "Fair," and "Poor," but only as "Good" and "Poor." Good results were those which represented 75 per cent or more of improvement while the result was described as poor if the parent reported that the child was not definitely improved. As a matter of fact, with few exceptions, these children were markedly improved or they were not improved at all. Those listed in this table under the heading "Dust" received no treatment other than oral dust therapy save for whatever medicinal treatment might be required for temporary relief. They were not instructed to buy mattress covers nor to do any unusual cleaning of the bed room in which the child slept. Feather pillows were not covered or removed unless the child was believed also to be sensitive to feathers.

Table II divides the good results of dust treatment and treatment with dust plus some other antigen into three groups based on the duration of the treatment for which we have satisfactory records. Since a considerable number of these were under treatment more than six months we believe that seasonal variations will not explain the good results obtained.

Table III shows the results of treatment according to the antigen involved and the type of allergic manifestations.

Table IV details the results at different age levels.

Table V shows the result of hypodermic therapy in those who failed to get relief by the oral method. Of those sensitive to dust, 77 of the 84 failures were treated hypodermically which accounts for the different total used in this table.

TABLE I. RESULT OF ORAL THERAPY

	DUST	GRASS	RAGWEED	TREES	TOTAL
Good	95	13	12	4	124
Poor	84	7	5	1	97

TABLE II. DURATION OF TREATMENT IN THOSE GETTING GOOD RESULTS

	1-3 MONTHS	3-6 MONTHS	OVER 6 MONTHS	TOTAL
Dust	36	15	53	104
Dust and other allergen	30	11	26	67

TABLE III. RESULTS OF TREATMENT ACCORDING TO THE ANTIGEN

	DUST		DUST AND OTHER		GRASS		RAGWEED		TREES	
	G	P	G	P	G	P	G	P	G	P
Asthma	52	47	42	4	5	3	6	3	1	0
Hay Fever	43	37	25	2	8	4	6	2	3	1

G=Good. P=Poor.

TABLE IV. RESULTS AT DIFFERENT AGE LEVELS

AGE IN YEARS		UNDER 1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-13	TOTAL
Dust	G	1	2	5	12	11	10	8	14	7	11	14	95
	P		4	7	11	11	11	8	12	5	4	11	84
Grass	G			1		2	3	1	1	2	2	1	13
	P				1		1	2			2	1	7
Ragweed	G				2	1	3	3	1		1	1	12
	P			1		1	1	1			1		5
Trees	G				1	1	1			1			4
	P					1							1

G=Good. P=Poor.

TABLE V. RESULT OF HYPODERMIC TREATMENT IN THOSE NOT RELIEVED BY ORAL THERAPY

	GOOD	POOR	NO REPORT	TOTAL
Dust	40	5	32	77
Grass	5	1	1	7
Ragweed	5	0	0	5

DISCUSSION

The evaluation of such a report as this is subject to the same criticism as is the evaluation of other forms of therapy such as, for example, the antihistaminic drugs. The report is based on the statement of the parent as it is interpreted by the physician and it must be admitted that there is some element of error involved. Since we have limited our division of results to "Good" and "Poor" and, since these designations are widely separated, we believe that the figures are reasonably dependable.

From Table III it is seen that the results with asthma and with perennial hay fever were approximately the same. The number sensitive to pollen alone and having seasonal symptoms was too small to justify definite conclusions.

It is an interesting observation, whose significance we would not attempt to appraise, that the results in children up to 6 years of age, treated with dust, were equally distributed between the "Good" and "Poor" while in the group between 6 and 13 years there is a significant difference. Whether this may be due to difference in absorption from the alimentary tract in the two groups we can only surmise. Again the number of patients treated with pollen is too small to be of significance.

The results of hypodermic therapy in patients who had failed to get relief from oral therapy would show that this procedure may always be available if and when the oral method fails.

SUMMARY

1. An unselected group of 402 children suffering from respiratory allergy was treated orally. Of these, the records on 294 were available for satisfactory evaluation of results. Of these, 221 had no treatment save oral therapy with a single antigen.

2. The method, as described, is simple, easy, and rapid and, when successful, to be preferred to hypodermic therapy.

3. The method was successful in more than one-half of those using it. Hypodermic therapy gave satisfactory relief in a large number of those who failed to get relief by the oral method.

REFERENCES

1. Touart, M. C.: Desensitization by Ingestion of Pollen Protein, N. Y. M. J. **116**: 199, 1922.
2. Black, J. H.: The Oral Administration of Pollen, J. Lab. & Clin. Med. **12**: 1156, 1927; **13**: 709, 1928.
3. Bernstein, T. B., and Feinberg, S. M.: Oral Ragweed Pollen Therapy; Clinical Results of Experiments on Gastrointestinal Absorption, Arch. Int. Med. **62**: 297, 1938.
   Forman, J.: Status of Oral Pollen Therapy of Hay Fever and Asthma, Ohio State M. J. **35**: 522, 1939.
   Zeller, M.: Oral Ragweed Pollen Therapy, J ALLERGY **10**: 579, 1939.
   Feinberg, S. M., Foran, F. L., Lichtenstein, M. R., Padnos, E., Rappaport, B. Z., Sheldon, J., and Zeller, M.: Oral Pollen Therapy in Ragweed Pollinosis; Cooperative Study, J. A. M. A. **115**: 23, 1940.
4. Thiberge, N. F.: The Use of Enteric Coated Pills in Allergic States, New Orleans M. & S. J. **94**: 390, 1942.
   Feinblatt, T. M., and Love, R.: Oral Pollen Absorption, Ann. Allergy **7**: 229, 1949.
   Urbach, E.: Desensibilisierung pollenallergischer Individuen auf gralem Wege mittels artspezifischer Pollenpeptone, Klin. Wehnschr. **10**: 534, 1931.
5. Black, J. H., and Shelmire, B.: The Urinary Proteose in Allergy, J. ALLERGY **5**: 373, 1934.
   Black, J. H.: Oral Administration of Ragweed Pollen, J. ALLERGY **10**: 156, 1939.
6. Levin, S. J., and Shulsky, L.: Serologic Changes after Oral Ragweed Pollen Therapy in Children, J. ALLERGY **13**: 1, 1941.
   Hecht, R., Mosko, M. M., Lubin, J., Sulzberger, M. B., and Baer, R. L.: The Absorption of Whole Ragweed Pollen from the Gastrointestinal Tract, J. ALLERGY **15**: 9, 1944.
7. Black, H.: The Treatment of Food Allergy, South. M. J. **35**: 771, 1942.
8. Schloss, O. M.: Allergy in Infants and Children, Am. J. Dis. Child. **19**: 433, 1920.

A TECHNIQUE FOR STUDYING THE NASAL ABSORPTION OF ALLERGENS IN HUMAN BEINGS*

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MANY investigators have demonstrated the passage of antigens through the nasal mucous membrane in animals. In human beings, this phenomenon has also been repeatedly demonstrated with a wide variety of substances, including insulin, pituitary extract, diphtheria toxoid and toxin, animal sera, pollens, silk, and others.

Different techniques[1-7] have been employed to demonstrate allergenic absorption. In most instances, the antigen was used in its natural state or in the form of a solution, a dry powder, or a dust. It was blown, sprayed, or instilled into the nose, or introduced on tampons. These techniques did not control the amount of material which came in contact with the nasal mucous membrane, nor the site or size of the absorptive surface. In fact, with most techniques, it was impossible to prevent the antigen, introduced into the nose, from reaching the lungs[19] or alimentary tract where absorption also occurred. Hence, these techniques were of limited value in quantitative studies on nasal absorption.

The present study was undertaken in order to find a suitable technique for studying the rapidity and amount of absorption from the nasal mucous membrane. The writers are of the opinion that an ideal technique for this purpose should possess all or most of the following features: (1) its performance should not be difficult; (2) it should furnish a readily accessible site in the nose for the test; (3) it should permit the use of a constant area of nasal mucous membrane as the absorbing surface; (4) it should permit the application of a constant amount of antigen to the absorbing surface; (5) it should furnish immediate and objective evidence of absorption; and (6) its performance should not be unpleasant to the subject.

Walzer and his associates[8-15] have shown that a passively sensitized cutaneous site will react following the systemic absorption of a related allergen through various portals of entry, including the mucous membranes of the alimentary and genitourinary tracts, the serous-lined body cavities, and the skin. Cohen, Ecker, Breitbart, and Rudolph[5] and Sulzberger and Vaughan[6] used variations of this technique to demonstrate the passage of inhaled antigen into the circulation.

A modification of the Walzer technique was adopted for the studies on nasal absorption about to be described.

TECHNIQUE

The principle involved in this technique was the one employed in studies on absorption from the alimentary tract.[8-12] The subjects to be tested were passively and locally sensitized with a suitable serum. After one or two days, the antigen

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was applied to the nasal mucous membrane. The onset of an urticarial reaction at the sensitized site indicated that absorption of the antigen had taken place.

The Sensitizing Serum:

The serum for sensitization was obtained from a patient who was markedly sensitive to peanut. Under sterile precautions, blood was obtained from the subject by venipuncture. It was allowed to coagulate and the clot to retract. The serum was then pipetted off, passed through a Seitz filter, and stored without preservative in the refrigerator.

The reagin titer of the serum was found to be 1:2048, by the dilution method of Coca and Grove.[16] Although the serum was used over a period of twenty-one months, its titer remained high and relatively constant.

Passive Local Sensitization:

Passive local sensitization of the subject to peanut was accomplished by the intracutaneous injection of 0.05 ml. of serum on the upper, outer aspect of the arm. To determine the most suitable dilution of the serum for these experiments, a series of subjects were sensitized, at different sites, with undiluted serum and with dilutions of 1:4 and 1:10. Although the serum was effective in all 3 dilutions, the 1:4 dilution proved most satisfactory and was adopted for the standard technique.

In order to prevent neutralization of the sensitizing reagins by ingested antigens, all types of nuts were eliminated from the subjects' diets for twenty-four hours preceding sensitization. In addition, peas, beans, and mustard were omitted from their diets, since reagins for these foods were also present in the serum.

The Positive Reaction at the Sensitized Site:

The absorption of peanut antigen into the circulation through the nasal mucous membrane was demonstrated by the onset, at the sensitized site, of a reaction consisting of erythema and wheal, usually accompanied by pruritus. The absorption rate was measured from the time of application of the antigen in the nose to the appearance of the first objective sign of a reaction at the sensitized cutaneous site.

Choice of Subjects:

Only nonatopic adults, free from nasal symptoms, were used in these experiments. Individuals with "colds" were rejected, as were those who had any previous nasal operations or frank evidence of chronic sinus infection. Sixty-eight of the subjects were males and 10 were females. Since nasal congestion frequently accompanies catamenia, female subjects were tested only during the intermenstrual period.

Skin tests, with an extract of peanut containing 0.01 mg. of total nitrogen per ml., were performed on all candidates several days before the experiment. Only negative reactors were accepted as subjects for these experiments.

Since it has been shown[17] that atopic individuals frequently fail to accept passive local sensitization normally, such subjects were not used in the present investigation. The study of nasal absorption in atopic individuals will be the subject of a future communication.

The Antigen:

The extract of peanut employed in these studies was freshly prepared according to the method of Coca and Milford,[18] and its total nitrogen content determined by the macro-Kjeldahl method. It was diluted to contain 0.05 mg. of nitrogen per ml.

Methods of Studying Absorption From Various Parts of the Nasal Mucous Membrane:

A. *A technique for studying absorption from the inferior meatus.*—With this technique, which was the first attempted, the solution of antigen was applied in the inferior meatus by means of a piece of cotton wrapped on a thin metal applicator. The size of the cotton pledget was approximately the same in each case. The pledget was moistened with that amount of antigen solution needed to wet the cottom without causing it to drip. The number of drops used was kept constant throughout the experiment. The cotton pledget containing the antigen was left in contact with the nasal mucous membrane until a reaction started at the sensitized cutaneous site.

At no time were anesthetics used in these experiments, since it was believed that their application to the nasal mucous membrane would affect the rate of absorption. The technique produced no untoward effects in the nose, and the mucous membrane at the site of application showed no gross changes.

Following the introduction of the cotton-tipped applicator moistened with antigen into the inferior meatus, the absorption reaction was regularly demonstrated. However, the presence of the probe under the inferior turbinate was painful to most subjects and this fact alone was sufficient to render this technique unsatisfactory.

B. *A technique for studying absorption from the middle meatus.*—After a number of trials with Technique A, the site of application was changed to the middle meatus. The technique remained the same, except that the metal probe was withdrawn after the pledget of antigen-soaked cotton had been tucked into the middle meatus.

From this site, absorption was also readily demonstrated. This technique was an improvement over the preceding one in that it was less painful and the site of application was more accessible. However, there were definite disadvantages connected with it. The anatomical structure of the middle meatus rendered it impossible to control the size of the absorptive area, an objection which applied to the preceding technique as well. The cotton pledget varied in its contacts with the lateral aspect of the turbinate and with the nasoantral wall. Moreover, the complex anatomy of the middle meatus, with sinus ostia opening into it, presented the possibility that antigen might enter the nasal accessory sinuses, thus increasing the absorptive area.

C. *A technique for studying absorption from the septum.*—In view of the many disadvantages associated with turbinates as a site of absorption, it was decided to investigate the possibility of utilizing the anterior inferior portion of the nasal septum for these studies. This area seemed desirable as a site for application of the allergen for several reasons. It would be readily accessible and entirely visible to the investigator. It would utilize a flat surface for the

test which would make it possible to measure accurately the area of mucous membrane which was being used as the absorptive surface.

Preliminary studies revealed that absorption from the medial wall of the nasal chamber occurred about as regularly as from the lateral wall. The size of the absorptive area on the septum was standardized by using small squares of compressed cotton,* measuring one square centimeter in area, for applying the antigen. These tabs were moistened with antigenic solution and, with the aid of a thumb forceps, were placed against the anterior portion of the septum. Contact between the tabs and the turbinates was avoided, since this would increase the absorptive surface. When exactly 0.25 ml. of solution was used, the tabs stayed in position on the septum without slipping and without losing their contents because of dripping. Because no pressure was exerted on the mucous membrane, this technique was painless. It presented no obvious disadvantages and seemed to provide suitable means for studying absorption from the nasal mucous membrane under standard conditions.

RESULTS OF TESTS FOR NASAL ABSORPTION

Preliminary studies with the 3 techniques described above were performed on 78 subjects. In all, 121 test trials were made, of which 113 gave positive results (Table I). Repeated trials on one subject gave consistently negative results which could not be accounted for. In other instances, failures were usually attributable to errors in technique or to faulty screening of subjects, resulting in the testing of atopic individuals. When such errors were recognized, the data on these subjects were omitted from the protocols, even when positive results were obtained. The influence of atopy on absorption from the nose will be the subject of another communication.

TABLE I. ABSORPTION OF PEANUT ANTIGEN FROM NASAL SEPTUM AND MEATI

	INFERIOR MEATUS			MIDDLE MEATUS			NASAL SEPTUM		
DILUTION OF SERUM	NO. OF CASES TESTED	NO. OF CASES POSITIVE	AVERAGE ABSORPTION RATE (MIN.)	NO. OF CASES TESTED	NO. OF CASES POSITIVE	AVERAGE ABSORPTION RATE (MIN.)	NO. OF CASES TESTED	NO. OF CASES POSITIVE	AVERAGE ABSORPTION RATE (MIN.)
Undiluted	6	6 (100%)	11.8	14	13 (92%)	11.7	4	3 (75%)	13.6
1:4	19	19 (100%)	12.4	15	14 (93%)	15.6	34	33 (97%)	14.1
1:10	13	13 (100%)	14.3	12	11 (92%)	16.3	4	1 (25%)	

EFFECT OF SERUM DILUTION ON ABSORPTION TIME

The effects of dilution of the sensitizing serum on absorption time are best illustrated in the studies of absorption from the middle meatus. A sufficient number of tests were made at this site with the 3 strengths of serum to permit comparisons of the results (Table II). Using the same technique throughout, 14 tests were made with the undiluted serum, 15 with the 1:4 dilution, and 13 with the 1:10 dilution. There was one failure with each dilution. With undiluted serum, the absorption times ranged from 6 to 18 minutes with an

*"Cottonoid," Johnson & Johnson Company, New Brunswick, N. J.

average of 11.7 minutes (Tables I and II). The 1:4 dilution of serum yielded absorption rates ranging from 8 to 29 minutes, with an average of 15.6 minutes. The absorption rates for the 1:10 dilution ran from 9 to 31 minutes, with an average of 16.3 minutes. Hence, dilution of the sensitizing serum appeared to lengthen the absorption rate to some degree.

TABLE II. ABSORPTION RATES OF PEANUT ANTIGEN WITH DIFFERENT DILUTIONS OF SENSITIZING SERUM

SERUM DILUTION	ABSORPTION RATES IN MINUTES FROM														
	INFERIOR MEATUS					MIDDLE MEATUS					SEPTUM				
Undiluted serum	2	9	12	13	13	6	7	9	9	9	11	14	16	F	
	22					11	11	13	14	15					
						15	16	18	F*						
Number of cases		6					14					4			
Average rate					11.8					11.7					13.6
Serum dilution 1:4	6.5	7	8	8	9	8	9	9	10	14	9	10	10	11	11
	9.5	10	10	11	12	14	15	15	16	16	11.5	12	12	12	12.5
	12.5	13	13	14	14.5	18	18	28	29	F	13	13	13.5	13.5	14
	16	18	21	23	F						14	14	14	14	14.5
											15	15	15	15	15
											15.5	16	16	17	18
											18	19	23	F	
Number of cases		20					15					34			
Average rate					12.4					15.6					14.1
Serum dilution 1:10	7	9	9	9.5	11	9	10	13	14	15	15.5	F	F	F	
	12	14	14.5	15	15	15	15	18	19	21					
	21	23	27			31	F								
Number of cases		13					12					4			
Average rate					14.3					16.3					

*F indicates failure to manifest absorption reaction.

Similar results were obtained in the studies on absorption from the inferior meatus and from the septum (Tables I and II). The same conclusion was also derived from those studies in which each subject was sensitized with the 3 dilutions of serum (Table III).

Despite the fact that undiluted serum yielded the most rapid absorption rates, its use presented certain disadvantages. In some cases, the positive cutaneous reaction which it produced was quite severe and discomforting to the subject. Furthermore, the amount of serum available was limited and its use in the undiluted form would have exhausted the supply before these studies could be completed. For these reasons, a dilution of serum for sensitization seemed desirable for the standard technique. However, the 1:10 dilution could not be relied upon since the occasional failure to demonstrate the absorption reaction occurred most frequently with this dilution (Tables I, II, and III). Since absorption occurred almost as regularly and as rapidly with the 1:4 dilution as with the undiluted serum, it was decided to use the 1:4 dilution almost exclusively in the remainder of the preliminary experiments. For this reason, there is a paucity of data, pertaining to studies with the undiluted serum and the 1:10 dilution, in some of the categories charted in the tables.

EFFECT OF SITE OF ABSORPTION ON ABSORPTION RATE

The preliminary studies of absorption from the inferior and middle meati and from the septum were each made with different techniques. Despite this

fact, differences in the rates of absorption from the 3 sites were comparatively small (Table II).

With the 1:4 dilution, the absorption rates for the inferior meatus seemed, on the average, to be about 3.2 minutes faster than for the middle meatus (12.4 as compared to 15.6 minutes). With the 1:10 dilution of serum, the difference averaged only 2 minutes (14.3 as compared to 16.3 minutes). The septal rate of absorption with the 1:4 dilution averaged 14.1 minutes, which fell between the absorption rates for the inferior meatus (12.4) and the middle meatus (15.6).

TABLE III. ABSORPTION RATES WITH VARYING SERUM DILUTIONS IN THE SAME SUBJECTS

SITE OF APPLICATION OF ALLERGEN	SUBJECT	ABSORPTION TIME IN MINUTES		
		SERUM UNDILUTED	SERUM DILUTION 1:4	SERUM DILUTION 1:10
Middle meatus	33	15	15	no reaction
	34	18	18	31
	35	15	16	21
	37	9	9	9
	42	9	8	10
	43	7	9	14
	45	13	14	18
Septum	44	16	16	no reaction
	45	13	14	18
	46	14	15	no reaction
	48	11	12	
	139		14.5	15.5
Inferior meatus	83		7	7
	84		11	11
	85		9.5	9.5
	86		14.5	14.5
	87		18	27
	89		10	12
	90		14	15
	91		6.5	9
	92		10	15
	93		16	21
	94		12.5	14
	96		21	23
	97		9	9

In view of the fact that a cotton pledget placed in either the middle or inferior meatus made contact with a larger absorptive area than that covered by a cotton tab on the septum, the rate of absorption from the septum compared favorably with the rates from the meati. Hence, the technique using the septum furnished information which appeared to be fairly representative of the respiratory nasal mucous membrane as a whole.

UNIFORMITY OF RESULTS OBTAINED WITH SEPTAL TECHNIQUE

With the recommended septal technique, the rate of nasal absorption in a given individual proved to be fairly constant. In connection with subsequent studies on nasal absorption in atopic subjects, it was necessary to make repeated use of several normal subjects as controls. It was found that the rate of absorption from the nasal septum in a given control, tested at intervals of several weeks or months, varied in repeated trials by only a few minutes. In

one subject, the absorption rates of tests made at different times were 14, 11, 14.5, 15, and 15 minutes. In another subject they were 10, 12.5, and 11.5 minutes. In a third they were 16 and 14.5 minutes, and in a fourth, 13.5 and 15.5 minutes.

SUMMARY

1. A technique which employs the anterior portion of the nasal septum as the site of application of antigen is presented for the study of absorption of allergens through the nasal mucous membrane.

2. The ready accessibility and visibility of the septum, and its flat surface which permits the use of constant amounts of antigen on constant absorptive areas, are among the outstanding advantages of the technique.

3. With the recommended technique, the absorption rate from the septum seems to be fairly representative of that from the anterior part of the respiratory nasal mucous membrane.

4. With the recommended technique, absorption of peanut antigen from the septum was demonstrated in 33 out of 34 subjects tested.

REFERENCES

1. Petragnani, G.: Anaphylaxis and Anti-Anaphylaxis by Nasal Route, Policlinico **29**: 446, 1922.
2. Ratner, B., Jackson, H. C., and Gruehl, H. L.: Respiratory Anaphylaxis: Sensitization, Shock, Bronchial Asthma and Death Induced in a Guinea Pig by Nasal Inhalation of Dry Horse Dander, Am. J. Dis. Child. **34**: 23, 1927.
3. Ratner, B., and Gruehl, H. L.: Respiratory Anaphylaxis (Asthma) and Ricin Poisoning Induced with Castor Bean Dust, Am. J. Hyg. **10**: 236, 1929.
4. Ratner, B.: Experimental Asthma, Am. J. Dis. Child. **58**: 699, 1939.
5. Cohen, M. B., Ecker, E. E., Breitbart, J. R., and Rudolph, J. A.: Rate of Absorption of Ragweed Pollen Material From the Nose, J. Immunol. **18**: 419, 1930.
6. Sulzberger, M. B., and Vaughan, W. T.: Experiments in Silk Hypersensitivity and the Inhalation of Allergens in Atopic Dermatitis, J. ALLERGY **5**: 554, 1934.
7. Simon, F. A., and Rackemann, F. M.: Development of Hypersensitiveness in Man, Absorption of Antigen Through Nasal Mucous Membrane, J. ALLERGY **5**: 451, 1934.
8. Walzer, M.: Studies in Absorption of Undigested Proteins in Human Beings. I. A Simple Direct Method of Studying the Absorption of Undigested Protein, J. Immunol. **14**: 143, 1927.
9. Walzer, M.: Absorption of Allergens, J. ALLERGY **13**: 554, 1942.
10. Brunner, M., and Walzer, M.: Absorption of Undigested Proteins in Human Beings: II. The Absorption of Unaltered Fish Proteins in Adults, Arch. Int. Med. **42**: 172, 1928.
11. Harten, M., Gray, I., Livingston, S., and Walzer, M.: Studies in Absorption of Undigested Protein. IX. Absorption From the Stomach and Esophagus, J. Lab. & Clin. Med. **27**: 54, 1941.
12. Gray, I., and Walzer, M.: Studies in Absorption of Undigested Protein in Human Beings. VIII. Absorption From the Rectum and a Comparative Study of Absorption Following Oral, Duodenal, and Rectal Administration, J. ALLERGY **11**: 245, 1940.
13. Rosenzweig, M., and Walzer, M.: The Absorption of Antigen from the Vagina and Cervix in Humans, Am. J. Obst. & Gynec. **45**: 286, 1943.
14. Walzer, A.: Cutaneous Absorption. I. A Direct Technique for Demonstrating the Percutaneous Absorption of Antigens, Arch. Dermat. & Syph. **41**: 692, 1940.
15. Baretz, L. H., Harten, M., and Walzer, M.: The Absorption of Protein from the Urinary Bladder, J. Urol. **50**: 71, 1943.
16. Coca, A. F., and Grove, E. F.: Studies in Hypersensitiveness. XIII. A Study of the Atopic Reagins, J. Immunol. **10**: 445, 1925.
17. Walzer, M., and Bowman, K.: Passive Local Sensitization in Atopic Individuals, Proc. Soc. Exper. Biol. & Med. **28**: 425, 1931.
18. Coca, A. F., and Milford, E. L.: The Preparation of Fluid Extracts and Solutions for Use in the Diagnosis and Treatment of Atopic Conditions, J. Immunol. **10**: 555, 1925.
19. Alexander, H. L., Becke, W. G., and Holmes, J. A.: Reactions of Sensitized Guinea Pigs to Inhaled Antigens, J. Immunol. **11**: 175, 1926.

CLINICAL AND EXPERIMENTAL EVALUATION OF THEPHORIN OINTMENT*

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IT IS beyond doubt that the histamine theory is a valuable aid in explaining certain allergic phenomena. On the basis of this concept, a search was started to find some substance which could block the action of histamine. Several attempts to counteract histaminic effect through the inhibitive action of a hypothetical enzyme, histaminase, proved a failure clinically. It was, therefore, an important step forward when Edelbacher, Jucker, and Baur[1] succeeded in counteracting the effect of histamine on the isolated intestine of the guinea pig with histidine, arginine, and cysteine. This was followed by the synthesis of a histamine antagonist by Halpern[2] and subsequently an array of new "antihistaminic" drugs was produced.

The oral utilization of these medicaments was soon followed by their trial topically in the treatment of pruritus and pruritic dermatoses. Sulzberger, Baer, and Levin,[3] Feinberg and Bernstein,[4] McGavack, Schulman, Schutzer, and Elias,[5] and Philip[6] have given good summaries of their experiences with Pyribenzamine and Benadryl in topical application, while Aaron, Peck, and Abramson[7] investigated the effect of Pyribenzamine iontophoresis. A class of compounds, chemically unrelated to these antihistaminic agents, was synthesized by Wenner and Plati[8] and one of these, Thephorin,† was found of value by Shelmire[9] and by Wooldridge and Joseph[10] who used it in an ointment base for the treatment of pruritic dermatoses. In view of their promising reports, we thought it of sufficient interest to give Thephorin Ointment a further clinical trial in itching and allergic dermatoses.

Also, in our hands, Thephorin Ointment had a definite antipruritic effect. Since Thephorin is an antihistaminic agent, it was logical to assume that this antipruritic effect was due to the ability of the drug to antagonize histamine. However, conflicting reports[5, 14] have appeared on the mode of action of another histamine antagonist, Benadryl, when applied topically. Therefore, experiments were undertaken to determine the mechanism responsible for the antipruritic effect of Thephorin Ointment.

Furthermore, studies on the action of Pyribenzamine in preventing erythema solare recently reported by Kurtin, Bierman, and Yontef,[11] Kline and Baer,[12] and Rubin, Beal, and Rothman[13] led us to investigate the possible protective action of Thephorin Ointment against the erythema produced by ultraviolet rays.

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†Thephorin is the "Roche" brand of phenindamine (Hoffmann-La Roche, Inc., Nutley, N. J.)

Accordingly, the work presented consists of three phases: the first covers our observations on the efficacy of this salve in the *treatment* of pruritic dermatoses; the second is concerned with investigations into the *mechanism* of the *antipruritic effect* of Thephorin Ointment; finally, the third phase deals with experiments concerning the *inhibitory action* of Thephorin Ointment on erythema solare.

The Thephorin Ointment employed consisted of Carbowax 1500 containing 5 per cent of the hydrogen tartrate of 2-methyl-9-phenyl-2,3,4,9-tetrahydro-1-pyridindene.

CLINICAL STUDY WITH THEPHORIN OINTMENT IN PRURITIC DERMATOSES

One hundred sixty-one patients with a variety of pruritic dermatoses were treated with Thephorin Ointment. The patients were instructed to apply the salve as often as was desired. The follow-up period ranged from 2 to 43 weeks. As is seen in Table I, listing the various diagnoses and summarizing the results, a total of 120 patients were benefited. In order to determine the antipruritic value, if any, of the Carbowax base alone, control assays on the therapeutic efficacy of a placebo ointment containing only Carbowax were performed on all subjects who claimed relief from Thephorin Ointment. Thus, 120 patients applied placebo salve either in alternation with the Thephorin Ointment or by the method of paired comparison,[15, 16] the base being applied to one part of the body and the Thephorin Ointment to another. Thirty-four of the subjects so tested obtained an approximately equal degree of relief from Carbowax and from Thephorin Ointment, but 86 were relieved only when they applied Thephorin Ointment. Table II portrays the situation, giving the types and numbers of patients who benefited from Thephorin Ointment only (on the left side of the dividing line), and listing the subjects who derived relief also from the placebo medication (on the right side of the dividing line).

The further clinical course of the 86 patients deriving benefit only from Thephorin Ointment was the following: Two patients with atopic dermatitis, after two months of treatment, obtained a clinical cure which was maintained without further therapy for an additional ten months—the period of observation. In addition, 3 of the patients with contact-type eczematous dermatitis obtained complete relief. The remaining 81 patients gained appreciable relief and, in many of these cases, dramatic relief of pruritus, although without complete and lasting cure, was obtained. Periodic reapplication of the medication was necessary in order to maintain freedom from itching. Despite the continued use, only one patient was observed who, after original improvement, later ceased to benefit from Thephorin Ointment.

In the total group of 161 patients, 4 cases of contact-type eczematous dermatitis were observed to develop under treatment. Of these, 3 developed the dermatitis at the site of application of both Thephorin Ointment and Carbowax base. The fourth patient was using Thephorin Ointment only. Patch tests were done and 3 of the patients were negative to both ointments; the fourth was positive to both the Thephorin and the base. The positive reactor

TABLE I. RESULTS OF TREATMENT WITH THEPHORIN OINTMENT IN ONE HUNDRED SIXTY-ONE PATIENTS WITH PRURITIC DERMATOSES

DIAGNOSIS	NUMBER OF PATIENTS	FEMALE	MALE	RESULTS								INCIDENCE OF SUCCESS (%)	INCIDENCE OF FAILURE (%)
				EXCELLENT		MODERATE		POOR OR NONE		WORSE			
				FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE		
Pruritus ani	55	40	15	25	10	4	2	9	2	2	1	74.5	25.5
Pruritus vulvae	12	12	--	6	--	4	--	2	--	--	--	83.3	16.7
Pruritus scroti	16	--	16	--	10	--	6	--	--	--	--	100.0	0.0
Nummular eczema	7	3	4	--	--	2	2	1	2	--	--	57.1	42.9
Contact dermatitis*	13	3	10	2	4	--	3	1	2	--	1	69.2	30.8
Atopic dermatitis	28	20	8	15	4	2	2	3	2	--	--	82.1	17.9
Circumscribed neurodermatitis	22	17	5	6	1	4	2	4	2	3	--	59.1	40.9
Generalized pruritus	7	4	3	1	1	1	1	1	1	1	--	57.1	42.9
Lichen planus	1	--	1	--	--	--	--	--	1	--	--	0.0	100.0
Total	161	99	62	55	30	17	18	21	12	6	2	74.5	25.5

*Subacute or chronic.

TABLE II. RESULTS OF CONTROL TESTS WITH NONMEDICATED CARBOWAX BASE IN ONE HUNDRED TWENTY PATIENTS DERIVING BENEFIT FROM THEPHORIN OINTMENT

DIAGNOSIS	TOTAL NO. OF PATIENTS	PATIENTS IMPROVED WITH THEPHORIN OINTMENT (NO.)	INCIDENCE OF IMPROVEMENT WITH THEPHORIN OINTMENT (%)	PATIENTS IMPROVED WITH THEPHORIN OINTMENT ONLY							PATIENTS IMPROVED WITH THEPHORIN OINTMENT AND WITH CARBOWAX BASE				
				DEGREE OF IMPROVEMENT											
				EXCELLENT		MODERATE									
				MALE	FEMALE	MALE	FEMALE	PATIENTS (NO.)	INCIDENCE OF IMPROVEMENT IN TOTAL NO. OF PATIENTS IMPROVED WITH THEPHORIN OINTMENT (%)	INCIDENCE OF IMPROVEMENT IN TOTAL NO. OF PATIENTS TREATED (%)	MALE	FEMALE	PATIENTS (NO.)	INCIDENCE OF IMPROVEMENT IN TOTAL NO. OF PATIENTS IMPROVED WITH THEPHORIN OINTMENT (%)	INCIDENCE OF IMPROVEMENT IN TOTAL NO. OF PATIENTS TREATED (%)
Pruritus ani	55	41	74.5	6	23	2	4	35	85.3	63.6	4	2	6	14.6	10.9
Pruritus vulvae	12	10	83.3	--	4	--	2	6	60.0	50.0	--	4	4	40.0	33.3
Pruritus scroti	16	16	100.0	8	--	3	--	11	68.8	68.6	5	--	5	31.2	31.2
Nummular eczema	7	4	57.1	--	--	1	1	2	50.0	28.6	1	1	2	50.0	28.5
Contact dermatitis	13	9	69.2	2	1	--	1	4	44.4	30.8	4	1	5	55.5	38.4
Atopic dermatitis	28	23	82.1	3	13	1	2	19	82.6	67.9	2	2	4	17.4	14.2
Circumscribed neurodermatitis	22	13	59.1	--	6	--	--	6	46.2	27.3	1	6	7	53.8	31.8
Generalized pruritus	7	4	57.1	--	1	1	1	3	75.0	42.8	1	--	1	25.0	14.3
Lichen planus	1	--	0.0	--	--	--	--	--	0.0	0.0	--	--	--	0.0	0.0
Total	161	120	74.5	19	48	8	11	86	71.6	53.4	18	16	34	28.3	21.1

to Thephorin and Carbowax tests, however, also showed sensitivity to a great many other therapeutic ointments and ointment bases. Five other patients developed side effects necessitating discontinuance of therapy. One complained of excessive dryness of the skin produced by both the base and the Thephorin Ointment; one developed pustules at the site of application of Thephorin Ointment; 3 noted a marked burning sensation. Thus, in all, 9 patients of 161 (5.6 per cent) developed reactions which necessitated cessation of therapy. Of these reactions, 4 were of a serious nature, but only in one instance was the reaction due to the development of sensitization to Thephorin Ointment.

INVESTIGATION INTO THE MECHANISM OF THE ANTIPRURITIC EFFECT OF THEPHORIN OINTMENT

Into the flexor surface of the forearm of each of 10 subjects, 0.1 c.c. of a freshly prepared histamine dihydrochloride solution (1:100,000) was injected intradermally. Ten minutes later, the wheals and flares were outlined with ink. The two largest diameters were measured and multiplied, the resultant figure being used as the standard for comparison. Four other sites were chosen on the flexor surface of the forearms of the same subjects. Two of these sites on each person were inuncted for two minutes with Thephorin Ointment and the nonmedicated Carbowax base, respectively. The salves were then allowed to remain in place for an additional three minutes. At this time, the ointments were removed and 0.1 c.c. of the histamine dihydrochloride solution (1:100,000) injected intradermally in the center of the areas of application. Ten minutes after the injection, the wheals and flares were outlined and measured as described for the control site. At the fourth and fifth sites, the histamine solution was injected first; five minutes later, Thephorin Ointment was applied on one and the Carbowax base on the other remaining site of each subject. Again, both ointments were lightly rubbed into the skin for two minutes and then permitted to remain in place for an additional three minutes. At the end of this time, that is, ten minutes after the injection, the ointments were removed and the wheals and flares measured. The times required for disappearance of all 5 reactions were noted.

In recording the findings, the sizes of the wheals and flares produced by the control injections of histamine were marked as 100 per cent.

From Table III, recording the sizes of the reactions to each of the test injections in per cent of the sizes of the corresponding control, it is evident that Thephorin Ointment, when applied either before or after the injection of histamine, had no significant effect on the size of the wheal. However, as is seen in the same table, Thephorin Ointment produced a marked inhibition of the flare when placed upon the skin prior to the injection of histamine. In Subject 3 this was manifested by a decrease in the intensity of the flare to a degree which rendered visual perception of the border difficult. The other 9 subjects showed a decrease of both the size and the intensity of the flare. No significant effect on the flare was noted when the Thephorin Ointment was applied after the injection of histamine, nor was the flare affected significantly by the Carbowax base. No appreciable difference between the duration of the control reactions and the test reactions was observed in any of the subjects.

TABLE III. CUTANEOUS RESPONSE TO INTRADERMAL INJECTION OF 0.1 C.C. OF A HISTAMINE DIHYDROCHLORIDE SOLUTION (1:100,000) WITHOUT OTHER MEDICATION, WITH THEPHORIN OINTMENT APPLICATION BEFORE AND AFTER INJECTION OF HISTAMINE, AND WITH CARBOWAX BASE BEFORE AND AFTER INJECTION OF HISTAMINE

SUBJECT (NO.)	CONTROL		THEPHORIN OINTMENT BEFORE		CARBOWAX BASE BEFORE		THEPHORIN OINTMENT AFTER		CARBOWAX BASE AFTER	
	WHEAL	FLARE	WHEAL	FLARE	WHEAL	FLARE	WHEAL	FLARE	WHEAL	FLARE
1	100	100	100.0	23.0	100.0	92.0	100.0	80.0	100.0	80.0
2	100	100	75.0	100.0	97.5	106.1	86.7	95.0	100.0	98.1
3	100	100	100 3	0	100.3	60.8	85.7	32 4	85 2	39.4
4	100	100	83.3	46.4	83.3	77.4	98 5	102.2	109.0	80.6
5	100	100	92.9	70.4	85.1	102.0	91.7	68.0	92 9	80.4
6	100	100	70.4	41.1	84.6	81.0	83.3	112.9	91.7	118.2
7	100	100	100.0	54.0	78 6	100.0	100.0	84.2	100 0	86.4
8	100	100	92.3	52.5	100.0	92 5	89.4	162.2	87.3	73.9
9	100	100	97.8	68.1	88.0	123.2	95.1	86.3	94.5	81.8
10	100	100	101.7	40.1	90.2	89.4	100.0	80.0	100.0	85.6
Average	100	100	91.37	49.56	90.76	92.44	93.04	84.32	96.06	82 94

EFFECT OF THEPHORIN ON PRODUCTION OF ERYTHEMA DUE TO ULTRAVIOLET LIGHT

Three dollar-sized areas were selected on the forearms of 4 healthy nurses. A thin coat of Thephorin Ointment was applied to one area and a 10 per cent boric acid ointment to the second area of each subject. No application was made to the third area. These sites were then exposed to the same, normally erythema-producing dose of ultraviolet light.

No erythema developed on the areas which had been covered with Thephorin Ointment; in contrast, deep erythema appeared on all control sites.

To determine the effect of Thephorin on erythema if the salve is applied after exposure, dollar-sized areas were selected on the forearms of four other subjects and exposed to ultraviolet light. Immediately following the exposure, Thephorin Ointment was applied to one area, 10 per cent boric acid ointment to the second, while no application was made to the third area of each of the subjects. Then, small dry dressings were applied and these, as well as the ointments, were removed after two and one-half hours. This latter experiment was repeated in 3 different subjects, but the ointments were permitted to remain in place for twenty-four hours.

The same degree of erythema developed on all sites of the subjects in both groups.

DISCUSSION

Undoubtedly, Thephorin Ointment has a very definite antipruritic effect, as evidenced by the moderate to excellent improvement of 74.5 per cent of the 161 patients studied. However, the Carbowax base alone proved to be equally soothing and antipruritic in 28.3 per cent of the patients deriving benefit from Thephorin Ointment. By the same token, 71.7 per cent of the subjects obtaining relief were benefited only when Thephorin Ointment was applied, but not when they received the nonmedicated Carbowax base. These results indicate clearly that in the great majority of cases, the relief is attributable to Thephorin and not to the base. Our findings are in agreement with those of Shelmire[9] who, after performing similar control tests, concluded that "far greater and more lasting relief of the pruritus was obtained from the salve containing Thephorin."

The fact that the majority of patients in our series were cases of long standing and had received a wide variety of therapy, including treatment with roentgen-rays, without appreciable benefit, is of importance in appraising the merits of Thephorin Ointment. Thus, of the 86 subjects who obtained relief from Thephorin Ointment, but not from the base, 26 had been ill for more than ten years, 26 for from five to ten years, 24 for from one to five years, and 10 for from four months to one year.

Side effects necessitating discontinuance of Thephorin occurred in 5.6 per cent of the patients treated with the salve. However, the reactions were of a serious nature in only 2.5 per cent of the entire series and the sensitizing index of Thephorin Ointment was found to be as low as 0.6 per cent. This approximates the same order of magnitude as the skin-sensitizing index of 1.3 per cent reported by Shelmire.[9] These findings prove the great safety of Thephorin Ointment.

The fact that Thephorin, when applied topically in an ointment base, has no significant effect on histamine-induced wheal formation suggests that the antipruritic effect of the salve may not be based on the antihistaminic action of the drug.

However, the ability of Thephorin to inhibit the flare produced by histamine suggests two other possible mechanisms. The flare is believed to result from the action of histamine in "stimulating the sensory endings of the skin and bringing about arteriolar dilatation through the mechanism of the axon reflex."[17] It appears likely therefore that Thephorin acts either on the sensory nerve endings or at the other end of the axon reflex. It has been pointed out that the flare is absent if the nerve endings in the skin have degenerated[18] or if the skin has been anesthetized.[18, 20] Hence, the inhibition of the flare produced by Thephorin Ointment may be due to the local anesthetic action of the drug. That it possesses a local anesthetic effect has been shown by Lehmann[21] who produced deep anesthesia of the rabbit's cornea with a one per cent solution of Thephorin.

It has been suggested by Dale that the flare which is the result of an axon reflex may be due to the release of acetylcholine at the nerve endings, the acetylcholine being held responsible for the arteriolar dilatation.[19] Hence, a second mechanism by which Thephorin inhibits the flare might be by means of an anticholinergic action. The anticholinergic action of Thephorin has been demonstrated by Lehmann, Randall, and Hagan,[22] who found it fairly potent in abolishing spasm of the small intestine produced by acetylcholine. In order to determine whether or not the inhibition of the histamine flare by Thephorin Ointment might be related to the anticholinergic action of the drug, the following experiments were carried out. One-hundredth mg. of mecholyl in 0.05 c.c. of normal saline was injected intradermally in the flexor surface of the forearm of each of 7 subjects. Five minutes after the injection, the resultant wheal and flare were outlined and measured as described for measuring the histamine reaction. On the other forearm of each subject, Thephorin Ointment was inuncted for two minutes and then permitted to remain in place for an ad-

ditional three minutes. At the end of this time, the ointment was removed and, in the center of the area of application, 0.01 mg. of mecholyl in 0.05 c.c. of physiologic saline was injected. Five minutes after injection the wheal and flare were measured. The time required for the disappearance of each of the reactions was noted.

It was found that in a given case the size of the wheal and flare following the injection of mecholyl intradermally did not differ significantly from the size of the wheal and flare which occurred when Thephorin Ointment had been applied prior to the injection. Similarly, the ointment did not affect the duration of reaction. These findings indicate that the inhibition of the histamine flare cannot be attributed to an anticholinergic action of Thephorin. Therefore, the most plausible explanation for the antipruritic effect of the salve is the local anesthetic action of Thephorin.

From the experiments constituting the third phase of our work, it was concluded that Thephorin Ointment, applied prior to the exposure to ultraviolet light, prevents erythema, but that the salve has no protective effect if it is applied after exposure or prior to the appearance of the erythema.

Kurtin, Bierman, and Yontef[11] found that areas of the skin treated with Pyribenzamine applied by iontophoresis prior to exposure to ultraviolet light failed to develop erythema, whereas control sites did show the expected erythema, edema, and vesiculation. On the basis of these findings, they concluded that the ultraviolet erythema is due to release of a histamine-like substance in the skin and that Pyribenzamine, by counteracting this substance, prevents the appearance of erythema.

However, Kline and Baer[12] showed that the action of Pyribenzamine was that of a screening agent. Our observations with Thephorin are in agreement with those of Kline and Baer and showed that Thephorin also, when used locally, inhibits erythema due to ultraviolet light, not by virtue of its antihistaminic action, but as a result of its ability to screen out those wave lengths responsible for the production of erythema.

The inhibition of erythema solare by application of Thephorin Ointment prior to exposure to ultraviolet rays point to the usefulness of the salve in preventing sunburn.

SUMMARY

1. Thephorin Ointment was evaluated clinically in 161 patients with a variety of pruritic dermatoses. Of these, 120, or 74.5 per cent, were helped. Thirty-four of those benefited obtained equal relief from the Carbowax base. In 86 patients, however, the subsidence of pruritus was unquestionably due to Thephorin since their itch was not relieved with the Carbowax alone. The antipruritic effect was best in cases of atopic dermatitis, and of pruritus ani, vulvae, and scroti. The comparatively high incidence of success and the low sensitizing index (0.6 per cent) warrants the keeping of Thephorin Ointment in the dermatotherapeutic armamentarium. However, the salve should be used with the realization of the occurrence of 2.5 per cent of contact-type reactions.

2. Experimental evidence is presented that Thephorin Ointment has no significant effect on histamine-induced wheal formation, but that it markedly inhibits the histamine flare. This suggests that Thephorin acts upon the axon reflex. This action may be due to an anesthetic effect on the sensory nerve endings in the skin or to an antagonism to acetylcholine released on the arterioles at the nerve endings. Evidence is presented that favors the theory that the inhibition of the histamine flare must be attributed to the anesthetic action of Thephorin Ointment on the sensory nerve endings of the skin. It is inferred that its antipruritic effect is based on the same mechanism.

3. Thephorin Ointment inhibits erythema solare if it is applied prior to the exposure to ultraviolet light. No inhibition occurs if the salve is applied after exposure.

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REFERENCES

1. Edelbacher, S., Jucker, P., and Baur, H.: Die Beeinflussung der Darmreaktion des Histamins durch Aminosauren, Ztschr. f. physiol. Chem. **247:** 63, 1937.
2. Halpern, B. N.: Les antihistaminics de synthese essais de chimiotherapie des etats allergiques, Arch. internat. de pharmacodyn. et de thérap. **68:** 339, 1942.
3. Sulzberger, M. B., Baer, R. L., and Levin, H. B.: Local Therapy with Pyribenzamine Hydrochloride, J. Invest. Dermat. **10:** 41, 1948.
4. Feinberg, S. M., and Bernstein, T. B.: Tripelennamine "Pyribenzamine" Ointment for the Relief of Itching, J. A. M. A. **134:** 874, 1947.
5. McGavack, T. H., Schulman, P., Schutzer, R., and Elias, H.: Effects of Topical Applications of Diphenhydramine Hydrochloride (Benadryl), Arch. Dermat. & Syph. **57:** 308, 1948.
6. Philip, A. J.: Treatment of Itching Dermatoses with an Ointment Containing Two Per Cent Diphenhydramine (Benadryl Hydrochloride), New York State J. Med. **49:** 1179, 1949.
7. Aaron, T. H., Peck, S. M., and Abramson, H. A.: Iontophoresis of Pyribenzamine Hydrochloride in Pruritic Dermatoses, J. Invest. Dermat. **10:** 85, 1948.
8. Wenner, W., and Plati, J. T.: Synthesis of Pyridindene Derivatives, (to be published).
9. Shelmire, B.: Topical Treatment With Thephorin, Postgrad. Med. **4:** 443, 1948.
10. Wooldridge, W. E., and Joseph, H. L.: Thephorin in the Treatment of Disseminated Neurodermatitis, J. Invest. Dermat. **11:** 93, 1948.
11. Kurtin, A., Bierman, W., and Yontef, R.: Inhibition of Erythema Solare in Normal Subject with Pyribenzamine, J. Invest. Dermat. **9:** 163, 1947.
12. Kline, P. R., and Baer, R. L.: Nature of Inhibition of Ultraviolet Erythema by Pyribenzamine, J. Invest. Dermat. **10:** 397, 1948.
13. Rubin, L., Beal, P. L., and Rothman, S.: A Method for Protection of Patients with Solar Urticaria, J. Invest. Dermat. **8:** 189, 1947.
14. Perry, D. J.: The Local Use of Benadryl Ointment, J. Invest. Dermat. **9:** 95, 1947.
15. Sulzberger, M. B., Baer, R. L., and Kanof, A.: Biologic Evaluation of Vehicles for External Application of Bal, Arch. Dermat. & Syph. **56:** 90, 1947.
16. Sulzberger, M. B., Baer, R. L., Kanof, A., and Lowenberg, C.: Methods for Rapid Evaluation of Beneficial and Harmful Effects of Agents Applied to Human Skin, J. Invest. Dermat. **7:** 227, 1946.
17. Best, C. H., and Taylor, N. B.: The Physiological Basis of Medical Practice, ed. 2, Baltimore, 1939, Williams & Wilkins Company, p. 441.
18. Ibid, p. 406.
19. Ibid, p. 444.
20. Fulton, J. F.: Howell's Textbook of Physiology, ed. 15, Philadelphia, 1947, W. B. Saunders Company, p. 830.
21. Lehmann, G.: Pharmacological Properties of a New Antihistaminic, 2-Methyl-9-Phenyl-2,3,4,9-Tetrahydro-1-Pyridindene (Thephorin) and Derivatives, J. Pharmacol. & Exper. Therap. **92:** 249, 1948.
22. Lehmann, G., Randall, L. O., and Hagan, E.: The Antihistamine, Anti-adrenaline and Anti-acetylcholine Action of Thephorin, Arch. Internat. de Pharmacodyn. et de thérap. **78:** 253, 1949.

STUDIES ON THE ANTIGENICITY OF BANANA, RAW AND DEHYDRATED*

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BANANA, a common food in tropical areas, is increasing in use as a popular item in the diet of people everywhere. Furthermore, banana is being utilized as an important factor in the dietary management of many diseases, for example, celiac disease, sprue, and other derangements of the gastrointestinal tract.

In spite of the fact that it is extensively eaten, very little is known of its antigenicity. In routine cutaneous testing, positive reactions to banana are not infrequent.[1, 2] Several authors have reported briefly on its etiology in acute urticaria,[3] bronchial asthma,[3] atopic eczema,[4, 5] allergic rhinitis,[6, 7] and gastrointestinal manifestations of allergy.[8]

Therefore, it seemed worth while to investigate the antigenic properties of this food. Extracts of raw, ripe banana and of commercially prepared dehydrated ripe banana were studied to determine their antigenicity in guinea pigs, and their allergenic properties in patients clinically sensitive to banana.

MATERIALS

Preparation of Extract of Fresh Banana.—The extracts used in these experiments were similar to those employed for routine intracutaneous testing in sensitive patients.

Seven ripe, 6-inch bananas (*Musa sapientum*) weighing 770 grams were mashed in a mortar and extracted under toluol at room temperature with 700 ml. of buffered saline solution. After 8 days, the uppermost brown-black layer and the toluol were discarded. The remaining substances were strained through gauze, filtered through paper, and passed through a Seitz filter. The clear, yellow-gray, sterile filtrate contained 0.53 mg. of nitrogen per ml., as determined by the macro-Kjeldahl method. This preparation was labeled B10.

Banana extract B11 was prepared in a similar fashion to B10 and contained 0.41 mg. of nitrogen per ml.

Preparation of Extract of Dehydrated Banana.—Fifty-seven Gm. of dehydrated banana,‡ representing 7 6-inch bananas *(Musa cavendishii),* were extracted with 700 ml. of buffered saline solution under toluol at room tem-

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‡The dehydrated banana used in these experiments is a canned preparation known as Kanana Banana Flakes, manufactured by Kannengiesser & Co., New York, N. Y. It is pure banana, processed to reduce moisture content with little loss of the nutritional and palatable properties of the original fruit.

According to information provided by the manufacturer, it is prepared as follows: The washed and peeled banana is reduced to a pulp and fed onto a revolving drum which is heated to approximately 103° C. Dehydration occurs in about 20 seconds. In some instances the process is shortened to 3 seconds at approximately 120° C. The resultant thin sheet is broken into flakes. The degree of heat used is just below that which would damage the vitamins or esters, caramelize the sugar, or affect color.

perature. On the eighth day, the toluol was discarded and the extract passed through paper and a Seitz filter. The sterile, yellow-green, clear filtrate contained 0.48 mg. of nitrogen per ml. and was labeled DB2.

Dehydrated banana extract DB3 was prepared in a similar fashion to DB2 and contained 0.41 mg. of nitrogen per ml.

A dialyzed extract was also prepared from defatted, dehydrated banana as follows: Fifty-seven Gm. of dehydrated banana were washed with ether, dried and ground to a fine powder. This was extracted under toluol at room temperature with 200 ml. of buffered saline solution. On the eighth day, the toluol was discarded and the remaining substances filtered through paper. The filtrate was dialyzed against physiologic saline solution for 3 days and then passed through a Seitz filter. The filtrate contained 0.37 mg. of nitrogen, and was labeled DB1.

EXPERIMENTS

Guinea pigs, weighing 195 to 430 grams, which had never been fed banana, were used in these experiments. Each animal was given a single intraperitoneal injection of extract for sensitization, and approximately 3 weeks later, an intracardiac injection for shocking purposes. After 4 to 16 days, those surviving this first intracardiac injection were then injected with the other extracts, as described below. Those succumbing were autopsied.

Series I.—Eleven guinea pigs were injected intraperitoneally with 3 to 5 ml. (1.23 to 2.12 mg. nitrogen) of banana extract (B10 or B11). Three weeks later they were injected intracardially with 0.25 to 1.0 ml. (0.14 to 0.53 mg. nitrogen) of the same extract with which they had been sensitized. All the animals reacted with anaphylactic shock. Nine died within 2 to 5 minutes, one in 10 minutes, and only one recovered (Table I).

Series II.—Six guinea pigs were injected with 3 to 5 ml. (1.25 to 2.05 mg. nitrogen) of banana extract (B11) (Table I).

A. Twenty-one days later, these were injected intracardially with 1.0 ml. (0.48 mg. nitrogen) of extract of dehydrated banana (DB2). None of the animals exhibited any evidence of sensitivity.

B. Two weeks later, these same animals were reinjected intracardially with 0.25 to 1.0 ml. (0.1 to 0.41 mg. nitrogen) of the same banana extract used for sensitization (B11). Four of the 6 guinea pigs died in acute anaphylactic shock.

Series III.—Five guinea pigs were given 3 to 5 ml. (1.44 to 2.40 mg. nitrogen) of extract of dehydrated banana (DB2) intraperitoneally (Table II).

A. Twenty-two days later, these were injected with 1.0 ml. (0.41 mg. nitrogen) of banana extract (B11) intracardially. None of the animals showed any evidence of sensitivity.

B. After another 16 days, these animals were reinjected intracardially with 1.0 ml. (0.41 mg. nitrogen) of extract of dehydrated banana (DB3). They showed no evidence of sensitivity.

Series IV.—Eleven guinea pigs were injected intraperitoneally with 1.0 to 3.0 ml. (0.35 to 1.44 mg. nitrogen) of extract of dehydrated banana (DB1 or DB2) (Table II).

A. After 21 to 24 days, 0.5 to 1.0 ml. (0.21 to 0.41 mg. nitrogen) of extract of dehydrated banana (DB1 or DB3) was given intracardially. No anaphylactic reactions were noted.

B. The same animals were reinjected intracardially with 0.4 to 1.0 ml. (0.16 to 0.53 mg. nitrogen) of banana extract (B10 or B11). None of the animals showed evidence of sensitivity.

Summarizing the results of these experiments, it is seen that:

Of the 17 guinea pigs sensitized with banana extract, 15 developed anaphylactic shock after receiving an intracardiac injection of the same extract. (Series I-A and Series II-B).

None of the 6 guinea pigs known to be sensitive to banana extract could be shocked by the injection of extract of dehydrated banana (Series II-A); nor could a potent banana extract shock 16 animals that were injected previously with extract of dehydrated banana (Series III-A and Series IV-B).

None of the 16 animals injected with the extract of dehydrated banana could be shocked with the homologous extract (Series III-B and Series IV-A).

OBSERVATIONS ON TWO PATIENTS SENSITIVE TO BANANA

In recent years, the following two cases of banana sensitivity came under the writers' observation.

CASE 1.—G. V., A 10-year-old boy, with ragweed hay fever and a positive family history for allergy, could eat banana without untoward reactions up to the age of 6 years. Since then, ingestion of as little as a teaspoonful of raw banana has been followed by protracted nausea, vomiting, and abdominal pain. On trial feeding, the child was able to eat commercial dehydrated banana with no ill effects.

CASE 2.—S. C., a 14-year-old girl, developed perennial allergic rhinitis at the age of 7, neurodermatitis 2 years later, and at the age of 12, bronchial asthma. Banana was known to cause severe abdominal cramps and diarrhea lasting two days. Contact of banana with the lips would cause local swelling and vesiculation. Other marked food sensitivities were present as well. Feeding of commercial dehydrated banana produced no ill effects.

Both patients gave marked (++++) intracutaneous reactions with banana extract diluted 1:10. However, extracts of dehydrated banana diluted 1:10 gave negative reactions.

Blood serum from patient G. V. was injected intracutaneously into two nonallergic substitutes for passive transfer studies.[9] The substitutes were placed on a diet free from banana and other foods to which the patient had shown positive cutaneous reactions. The sites were tested intracutaneously with diluting fluid and with extracts of dehydrated banana and of raw banana. The results are given in Table III. The presence of specific skin-sensitizing antibodies for banana was demonstrated. However, these antibodies did not react with extracts of dehydrated banana.

TABLE I. GUINEA PIGS INJECTED INTRAPERITONEALLY WITH BANANA EXTRACT AND CHALLENGED INTRACARDIALLY WITH BANANA OR DEHYDRATED BANANA EXTRACT

			INTRAPERITONEAL INJECTION			A. FIRST INTRACARDIAL INJECTION						B. SECOND INTRACARDIAL INJECTION					
SERIES	GUINEA PIG (NO.)	WEIGHT (GM.)	ANTIGEN	AMOUNT (ML.)	NITROGEN (MG.)	INTERVAL (DAYS)*	ANTIGEN	AMOUNT (ML.)	NITROGEN (MG.)	RESULT	TIME OF DEATH (MIN.)	INTERVAL (DAYS)*	ANTIGEN	AMOUNT (ML.)	NITROGEN (MG.)	RESULT	TIME OF DEATH (MIN.)
I	1	275	B10	4.0	2.12	21	B10	1.0	0.53	X	3						
	2	355	B10	4.0	2.12	21	B10	0.5	0.27	X	3						
	3	240	B10	4.0	2.12	22	B10	0.25	0.14	X	4						
	4	390	B10	4.0	2.12	22	B10	0.25	0.14	X	5						
	5	360	B10	3.0	1.59	21	B10	0.25	0.14	X	3						
	6	430	B10	3.0	1.59	22	B10	0.25	0.14	X	10						
	7	290	B11	5.0	2.05	21	B11	1.0	0.41	X	2						
	8	245	B11	5.0	2.05	22	B11	1.0	0.41	X	5						
	9	220	B11	3.0	1.23	22	B11	1.0	0.41	X	3						
	10	230	B11	3.0	1.23	22	B11	1.0	0.41	X	4						
	11	215	B11	3.0	1.23	22	B11	0.4	0.16	+							
II	12	200	B11	3.0	1.23	21	DB2	1.0	0.48	0		35	B11	0.25	0.10	0	
	13	200	B11	3.0	1.23	21	DB2	1.0	0.48	0		35	B11	1.0	0.41	X	3
	14	210	B11	5.0	2.05	21	DB2	1.0	0.48	0		35	B11	0.5	0.2	X	4
	15	200	B11	3.0	1.23	21	DB2	1.0	0.48	0		35	B11	0.15	0.06	0	
	16	205	B11	5.0	2.05	21	DB2	1.0	0.48	0		35	B11	0.8	0.33	X	4
	17	210	B11	5.0	2.05	21	DB2	1.0	0.48	0		35	B11	0.25	0.10	X	3

*Number of days after intraperitoneal injection.
B10, B11 = Banana extracts.
DB2 = Dehydrated banana extract.
X = Died in anaphylactic shock.
+ = Positive but not fatal reaction.

TABLE II. GUINEA PIGS INJECTED INTRAPERITONEALLY WITH DEHYDRATED BANANA EXTRACT AND CHALLENGED INTRACARDIALLY WITH BANANA OR DEHYDRATED BANANA EXTRACT

			INTRAPERITONEAL INJECTION			A. FIRST INTRACARDIAL INJECTION					B. SECOND INTRACARDIAL INJECTION				
SERIES	GUINEA PIG (NO.)	WEIGHT (GM.)	ANTIGEN	AMOUNT (ML.)	NITROGEN (MG.)	INTERVAL (DAYS)*	ANTIGEN	AMOUNT (ML.)	NITROGEN (MG.)	RESULT	INTERVAL (DAYS)*	ANTIGEN	AMOUNT (ML.)	NITROGEN (MG.)	RESULT
III	18	270	DB2	5.0	2.40	22	B11	1.0	0.41	0	38	DB3	1.0	0.41	0
	19	220	DB2	3.0	1.44	22	B11	1.0	0.41	0	38	DB3	1.0	0.41	0
	20	205	DB2	3.0	1.44	22	B11	1.0	0.41	0	38	DB3	1.0	0.41	0
	21	250	DB2	5.0	2.40	22	B11	1.0	0.41	0	38	DB3	1.0	0.41	0
	22	200	DB2	3.0	1.44	22	B11	1.0	0.41	0	38	DB3	1.0	0.41	0
IV	23	310	DB1	1.0	0.35	21	DB1	1.0	0.35	0	28	B10	1.0	0.53	0
	24	210	DB1	1.0	0.35	24	DB1	1.0	0.35	0	28	B10	1.0	0.53	0
	25	300	DB1	2.0	0.70	21	DB1	1.0	0.35	0	28	B10	1.0	0.53	0
	26	300	DB1	2.0	0.70	24	DB1	1.0	0.35	0	28	B10	1.0	0.53	0
	27	340	DB1	3.0	1.05	21	DB1	1.0	0.35	0	28	B10	1.0	0.53	0
	28	200	DB2	3.0	1.44	24	DB3	1.0	0.41	0	35	B11	1.0	0.41	0
	29	195	DB2	3.0	1.44	24	DB3	0.5	0.21	0	35	B11	0.4	0.16	0
	30	260	DB2	2.0	0.96	21	DB3	1.0	0.41	0	35	B11	1.0	0.41	0
	31	320	DB2	2.0	0.96	21	DB3	0.6	0.25	0	35	B11	1.0	0.41	0
	32	300	DB2	2.0	0.96	21	DB3	1.0	0.41	0	35	B11	1.0	0.41	0
	33	235	DB2	2.0	0.96	21	DB3	0.6	0.25	0	35	B11	1.0	0.41	0

*Number of days after intraperitoneal injection.
B10, B11 = Banana extracts.
DB1, DB2, DB3 = Dehydrated banana extracts.

TABLE III. PASSIVE TRANSFER STUDIES ON BLOOD SERUM OF PATIENT SENSITIVE TO BANANA (G. V.)

ANTIGEN	REACTION	
	SENSITIZED SITE	CONTROL SITE
Diluting fluid	−	−
B10	++++	+
B10	+++	+
B11	+++	−
B11 1:10	++++	+
B11 1:100	++	±
DB1	−	−
DB1	−	−
DB2	±	±
DB2	±	±
DB2	±	±
DB3	+	+
DB3 1:10	−	−

Negative	−	Slight moderate	++
Slight negative	±	Moderate	+++
Slight	+	Marked	++++

B10, B11 = Banana extracts.
DB1, DB2, DB3 = Dehydrated banana extracts.

DISCUSSION

Our experiments prove that banana extract has anaphylactogenic properties, producing shock in 15 of the 17 guinea pigs sensitized. We found that quantities of extract containing as little 1.23 mg. of nitrogen could sensitize a guinea pig, and as little as 0.14 mg. of nitrogen could shock one.

Raw, ripe banana contains 1.2 per cent protein, 23.0 per cent carbohydrate, 0.2 per cent fat, and 75.6 per cent water, in addition to minerals and vitamins.[10] The extracts used in our experiments were analyzed for the total nitrogen content only. This was taken as the measure of the concentration of the extract and was presumed to approximate its antigenicity. The possible antigenic role of the other constituents, particularly carbohydrate, awaits further investigation.

The dehydrated banana used in our experiments contains 6.8 per cent proteins, 83.4 per cent carbohydrates, 1.3 per cent fat, minerals and vitamins, and only 5.5 per cent moisture.* The extract prepared from it contained approximately the same amount of total nitrogen per ml. as the banana extract. With the technique used, the extract of dehydrated banana was devoid of any sensitizing or shocking properties. Guinea pigs could not be sensitized with as much as 2.4 mg. of nitrogen of extract of commercial dehydrated banana (contained in 5.0 ml., which is the maximum that could be injected intraperitoneally at one time). As much as 0.41 mg. of nitrogen of the same extract failed to produce shock. This would seem to indicate that in the process of dehydration of the banana by heat, the antigen is so altered that its antigenic properties are destroyed.

It has been observed that food atopens such as milk and cereals could sensitize and shock guinea pigs. However, these same foods when processed by heat, as during cooking, canning, or dehydration, showed a marked reduction in their anaphylactogenic properties.[11-14]

*Analysis as accepted by the Council on Foods and Nutrition of the American Medical Association.

The two cases presented showed immediate allergic gastrointestinal manifestations from ingestion of raw banana. That banana is a strong atopen was further demonstrated by direct intracutaneous testing and passive transfer studies. The reagins could not be demonstrated by using the extract of dehydrated banana. We assume, therefore, that this extract is devoid of active atopens. Moreover, the fact that the patients could tolerate considerable quantities of dehydrated banana attests further to its low allergenicity. One may assume that there is a definite place for this product in the hypoallergenic diet.

SUMMARY

1. Guinea pigs can be sensitized with an extract of raw banana, but not with a similarly prepared extract of the same nitrogen content of heat-processed, dehydrated banana flakes.

2. Guinea pigs specifically sensitized to the raw fruit can be shocked with the extract of raw banana, but not with the extract of the dehydrated banana.

3. Guinea pigs injected with dehydrated banana cannot be shocked with banana or dehydrated banana.

4. Two atopic children, clinically sensitive to banana, could ingest dehydrated banana without allergic manifestations. Although sensitive by cutaneous reactions to raw banana extract, both showed negative reactions to dehydrated banana. In one, the results of these cutaneous reactions were corroborated by passive transfer studies.

REFERENCES

1. Rowe, A. H.: Clinical Allergy, Philadelphia, 1937, Lea & Febiger, pp. 545, 547.
2. Feinberg, S. M.: Allergy in Practice, Chicago, 1946, The Year Book Publishers, Inc., p. 468.
3. Feinberg, S. M.: Allergy in General Practice, Philadelphia, 1934, Lea & Febiger, p. 69.
4. Strobl, A., and Wasitzky, A.: Untersuchungen über die Alimentaire Allergie des Ekzemkindes mit besonder Berucksichtigung der Eiklaremfindlichkeit, Monatschr. f. Kinderh. **54**: 53, 1932.
5. Ratner, B.: A Possible Causal Factor of Food Allergy in Certain Infants, Am. J. Dis. Child. **36**: 277, 1928.
6. Lindsay, J. C.: Food Anaphylaxis, Canad. M. A. J. **16**: 58, 1926.
7. Goodale, J. L.: Diagnosis and Management of Vasomotor Disturbances of the Upper Air Passages, Boston M. & S. J., **175**: 181, 1916.
8. Rowe, A. H.: (reference 1) p. 586.
9. Walzer, M.: "Skin Testing," in: Cooke, Robert: Allergy in Theory and Practice, Philadelphia, 1947, W. B. Saunders Company, p. 508.
10. Sherman, H. C.: Chemistry of Food and Nutrition, ed. 7, New York, 1946, The Macmillan Company, p. 261.
11. Cutler, O. I.: Antigenic Properties of Evaporated Milk, J. A. M. A. **92**: 964, 1929.
12. Lewis, J. H., and Hayden, H. C.: Effect of Heat on the Antigenic Properties of Milk, Am. J. Dis. Child. **44**: 1211, 1932.
13. Ratner, B., and Gruehl, H. L.: Anaphylactogenic Properties of Milk, Am. J. Dis. Child. **49**: 287, 1935.
14. Ratner, B., and Gruehl, H. L.: Anaphylactogenic Properties of Certain Cereal Foods and Breadstuffs, Am. J. Dis. Child. **57**: 739, 1939.

PENICILLIOSIS AND HYPERSENSITIVENESS TO PENICILLIN*

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ACCIDENTS due to sensitization to penicillin are daily increasing in number as the use of penicillin grows. The industrial purifying of this drug, and especially the preparation of crystallized forms, has reduced hypersensitiveness phenomena to those intrinsically bound up with true sensitization to certain molecular groups of penicillin itself. It was consequently thought that the antigen of this might be in some ways related to that present in certain fungi which are agents of human mycoses (especially trichophytin)[1, 2] which might indirectly sensitize the subject to penicillin. The finding of a history of previous mycosis in some patients sensitized to penicillin would seem to confirm this assumption. In other cases, sensitization has been brought about by penicillin itself, the drug being well tolerated once or twice and urticaria arising after subsequent administrations.

The study of a patient who was gradually sensitized to penicillin which induced intense urticaria in him, persisting for a long time after the drug was given up, led us to wonder whether this patient might not have the mold, Penicillium, in some part of his system and whether the penicillin had sensitized him to the metabolic products of the fungus. The reason for this suspicion was that we had already previously found that the culture in Sabouraud's medium of feces from a patient who proved hypersensitive to penicillin since the very first administrations of the drug produced abundant colonies of Penicillium. This discovery, of which we were then unable to make any use, led us to think that the other patient with penicillinic urticaria and spontaneous urticaria after the stopping of the drug might also prove to have Penicillium in his system. As he had a lung abscess, we made cultures of both sputum and feces. In both, we found abundant colonies of two Penicillia, and both this case and the previous one seem to point to the existence, at least in some subjects, of a mechanism of sensitization to penicillin through the Penicillium themselves, of which we have found no mention so far in the literature we have been able to peruse.

We think a summary exposition of the clinical case may be interesting:

CASE REPORT

M. C., a 50-year-old man, was a medical assistant. He had an allergic family history (urticaria, eczema) and hematic eosinophiles (9 per cent). For the last 20 years he has had a pulmonary abscess left over by a hydatidic cyst. He has had several evolutive fever incidences, the last three of which were treated with intramuscular penicillin. The first time he was given 1,200,000 units in 4 days, and 48 hours after the end of the treatment generalized urticaria set in, lasting several days. Later, for another febrile respiratory episode, penicillin was again injected and he had another attack of urticaria 24 hours after the drug was stopped; 6 months later still more injections had to be given him, and after the

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first one of 10,000 units of penicillin, an urticarial wheal appeared on the spot of the injection and the eruption rapidly spread to the whole body. Since then, he has had urticaria spontaneously every day. His hypersensitiveness to penicillin is so marked that intradermic injection of 1 unit in 0.05 c.c. produces an intense reaction (pseudopodic).

From the analysis of this case it would seem that we may suspect, since the existence of Penicillium in the sputum and feces was ascertained, that the subject was not only sensitized to penicillin but also to the metabolic products of the Penicillium harbored in his respiratory and digestive systems. It was to be suspected that there was something in common between the drug and those parasitic fungi, and the first thing that occurred to us was the penicillin itself, assuming that the fungi in question were able to produce it; but this was a priori unlikely for the following reasons: A microscopic study of the sputum and feces revealed no structure suspect of mycelium; on the other hand, it is unlikely that, in the temperature conditions of the system, Penicillium can prosper. We had also to add to this the presence of abundant *Klebsiella pneumoniae* in the sputum and *Escherichia coli* in the feces, both of which are producers of penicillinase. But, furthermore, the study of these fungi, carried out by Dr. Bustinza in his laboratory at Madrid Botanical Garden, has proved that they do not produce any substance with antibiotic qualities, and this clinches the matter.

But the later course of the patient's trouble and the result of desensitizing treatment with an extract of the spores of his Penicillium, to which when previously tested he had reacted positively and intensely, proves the existence of something in common between penicillin and the substance present in the spores of those fungi. And indeed, progressive desensitization with the aforesaid extract has resulted in the disappearance of the urticaria and the patient's tolerance of penicillin, of which it has been possible to inject 1,000,000 units in one week without his experiencing any trouble.

Afterward, studying the feces of eight persons, of whom two are hypersensitive to penicillin and the other six can tolerate it, we have found Penicillium in the two hypersensitive ones and in one case in the nonsensitive group while investigation of the remaining five proved negative. Adding these two cases to those previously described we get four patients hypersensitive to penicillin with Penicillium found in the feces (and in one case in the sputum), and six nonsensitive ones, of whom only one had this fungus in his bowels.

These findings, which must go on being studied, put us on the track of a possible relation between allergy and penicillin and the presence of Penicillium in the system, and throw light on the possible etiological role of this fungus or of others in the production of urticarias which so far were held to be cryptogenetic.

SUMMARY

1. We describe two cases of penicillinic urticaria in which Penicillium was found in the feces and in one also in the sputum.

2. One of the patients went on having urticaria afterward, and this was attributed to the presence of the fungus in his system.

3. When this last-named patient was treated with an extract of the spores of his Penicillium his urticaria disappeared and he was desensitized to penicillin.

4. The idea is set forth, based on still scanty statistics, that this mechanism may be the cause of a good number of sensitizations to penicillin and the hypothesis is advanced that certain forms of urticaria which have so far been considered cryptogenetic may be due to the same mycotic sensitization.

REFERENCES

1. Surinyac, R.: Med. Klin. Barcelona **12**: 65, 1949.
2. Isler, H., and Karabadjakian: Acta. allerg. **5**: 297, 1948.
3. Sanchez-Cuenca, B.: Bol. Inst. Pat. Med., Prof. Maranon, Madrid, June, 1949.

AN APPARATUS FOR ASPIRATION OF POSTERIOR PHARYNGEAL SECRETIONS

Rubber-Tip Suction as an Aid to Obtaining Postnasal Mucoid Material*

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IN ATTEMPTING to demonstrate eosinophilic cells in routine smears of the nasal secretions we have developed an apparatus whose simplicity, inexpensiveness, and effectiveness would seem to warrant its description. This instrument has been designed and used with considerable success in carrying out diagnostic studies in a large number of pediatric patients.[1]

Construction of the apparatus was prompted by repeated failures to demonstrate eosinophilic cells in routine smears of nasal secretions obtained from sputum smears, smears of freshly swabbed secretions from the anterior nares, and smears of nasal discharge or swabbings from the posterior nasopharynx. Although clinical impressions of allergic reaction throughout the entire upper respiratory tract (and especially the sinuses) were confirmed in all patients by positive skin reactions to allergenic agents, the techniques enumerated above repeatedly gave positive tests for eosinophilic secretions in less than 15 per cent of patients studied. Such a small incidence of demonstrable eosinophilia in nasal secretions contrasted poorly with the studies of Hansel[2, 3] and others,[4] who report over 90 per cent positive smears obtained in serial studies of nasopharyngeal secretions taken from known allergic patients.

Accordingly we devised the apparatus illustrated in Fig. 1. It is assembled from a shortened 20 ml. Fisher pipette of 0.6 cm. bore, a small rubber bulb, and a small length of flexible rubber tubing of 0.7 cm. diameter. The soft rubber tip prevents injury to the pharynx, and the rubber sheath overlying the glass protects the stem from breakage incident to striking against teeth.

The instrument is utilized as follows: The physician, equipped with headlamp and two tongue depressors, inspects the posterior pharynx for site of the postnasal mucoid discharge. This is done usually in the morning about half an hour after the patient has awakened and when his sitting position has encouraged postnasal drainage of secretions from the overfilled sinuses. With tongue depressors gently depressing the tongue base, and so positioned against the teeth as to prevent impromptu closure of the mouth, the aspirator (with hand bulb compressed) is slipped quickly into the throat. Mucus is suctioned from the posterior pharyngeal wall into the pipette barrel and the apparatus is then promptly withdrawn.

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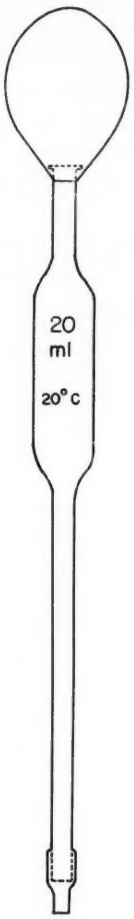

Fig. 1.—Aspirator for obtaining posterior pharyngeal secretions.

Thin smears of the secretions thus obtained are prepared at once by depositing a small glob of the mucoid matter on the surface of a clean glass slide, and spreading the material over the length of the glass much in the manner of preparing a blood film. When dry, the smear is stained, using either Mallory's[5] or Hansel's[6] solutions, and then the dry preparation is examined microscopically for evidence of the brightly colored clumps of eosinophilic cells whose presence denotes a "positive" smear.

REFERENCES

1. Price, W. C., and Solow, I. A.: Incidence of Nasal Eosinophilia in 120 Allergic Children. In preparation.
2. Hansel, F. K.: Allergy of the Nose and Paranasal Sinuses, St. Louis, 1936, The C. V. Mosby Company.
3. Hansel, F. K.: Diagnosis of Allergy of the Nose and Paranasal Sinuses, Arch. Otolaryng. **34**: 1152, 1941.
4. Rhodes, W. L.: Allergy and Infection of the Nose and the Paranasal Sinuses, M. Rec. **156**: 224, 1943.
5. Gradwohl, R. B. H.: Clinical Laboratory Methods and Diagnosis, St. Louis, 1943, The C. V. Mosby Company, pp. 943-944.
6. Gradwohl, R. B. H.: Clinical Laboratory Methods and Diagnosis, St. Louis, 1943, The C. V. Mosby Company, p. 1083.